

**EXHIBIT A**  
**PENDING CLAIMS CORRELATED**  
**WITH CLAIM NUMBERS IN SERIAL NO. 08/872,222**

**Former Claim 11:**

11. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein said phosphoinositide analogue incorporates one or more of the following modifying structural features:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; or
- (b) a reporter group or conjugand is incorporated in the fatty acyl or inositol residue;

wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**Former Claim 12:**

12. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(mono-phosphate) analogue.

**Former Claim 13:**

13. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(di-phosphate) analogue.

**Former Claim 14:**

14. The phosphoinositide analogue of claim 13, wherein said phosphoinositide analogue is a PtdIns(4,5)P<sub>2</sub> analogue.

**Former Claim 15:**

15. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(poly-phosphate) analogue.

**Former Claim 16:**

16. The phosphoinositide analogue of claim 11, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement.

**Former Claim 17:**

17. The phosphoinositide analogue of claim 16, wherein the 2-OH is rendered non-nucleophilic by derivatization.

**Former Claim 18:**

18. The phosphoinositide analogue of claim 17, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is alkyl, substituted alkyl or alkenyl.

**Former Claim 19:**

19. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form 2-OAc.

**Former Claim 20:**

20. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is CH<sub>3</sub>.

**Former Claim 21:**

21. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is  $\omega$ -amino-alkyl.

**Former Claim 22:**

22. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is N-substituted- $\omega$ -amino-alkyl.

**Former Claim 23:**

23. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is N,N-disubstituted- $\omega$ -amino-alkyl.

**Former Claim 24:**

24. The phosphoinositide analogue of claim 16, wherein the 2-OH is rendered non-nucleophilic by replacement.

**Former Claim 25:**

25. The phosphoinositide analogue of claim 24, wherein the 2-OH is rendered non-nucleophilic by replacement to form the 2-deoxyhalo or 2-dideoxyhalo phosphoinositide analogue.

**Former Claim 26:**

26. The phosphoinositide analogue of claim 25, wherein the 2-OH is rendered non-nucleophilic by replacement to form the 2-deoxyfluoro phosphoinositide analogue.

**Former Claim 27:**

27. The phosphoinositide analogue of claim 11, wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue.

**Former Claim 28:**

28. The phosphoinositide analogue of claim 27, wherein a reporter group is incorporated.

**Former Claim 29:**

29. The phosphoinositide analogue of claim 28, wherein the reporter group is a photoaffinity reporter group.

**Former Claim 30:**

30. The phosphoinositide analogue of claim 28, wherein the reporter group is a fluorescent reporter group.

**Former Claim 31:**

31. The phosphoinositide analogue of claim 28, wherein the reporter group is a spin probe reporter group.

**Former Claim 32:**

32. The phosphoinositide analogue of claim 28, wherein the reporter group is a radioactive label reporter group.

**Former Claim 33:**

33. The phosphoinositide analogue of claim 28, wherein the reporter group is a stable isotope label reporter group.

**Former Claim 34:**

34. The phosphoinositide analogue of claim 27, wherein a conjugand is incorporated.

**Former Claim 35:**

35. The phosphoinositide analogue of claim 34, wherein the conjugand is alkyl-C=O,  $\omega$ -NH<sub>2</sub>-alkyl-C=O,  $\omega$ -NH<sub>2</sub>-alkyl,  $\omega$ -thio-(alkyl-C=O) or  $\omega$ -thio-alkyl.

**Former Claim 36:**

36. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a polymer.

**Former Claim 37:**

37. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a chromatographic matrix.

**Former Claim 38:**

38. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a gold surface.

**Former Claim 39:**

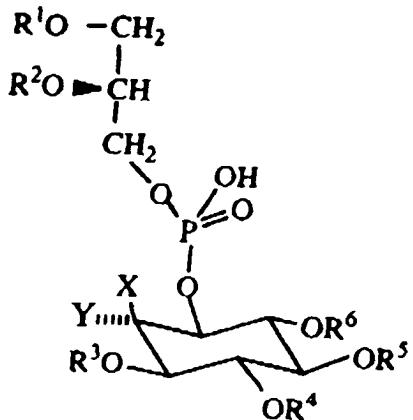
39. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a reporter group.

**Former Claim 40:**

40. The phosphoinositide analogue of claim 11, wherein one or both glycerol esters are replaced by ether bonds.

**Former Claim 66:**

41. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glycero-ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> is P(=O)(O-protecting group)<sub>2</sub>,

and wherein:

- (a) X = F, Cl, Br, OC(=O)R, OR, or P(=O)(O-protecting group)<sub>2</sub>, and Y = H; or  
X = Y = H; or
- (b) X = H, and Y = F, Cl, Br, OC(=O)R, OR, or P(=O)(O-protecting group)<sub>2</sub>; or  
X = Y = F or (=O);  
where R = alkyl, especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

- (d) R<sup>1</sup> = RC(=O) or R, R<sup>2</sup> = R'C(=O) or R'  
where R, R' = alkyl or alkenyl;

and wherein:

- (e) R<sup>3</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (f) R<sup>4</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (g) R<sup>5</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (h) R<sup>6</sup> = H, P(=O)(O-protecting group)<sub>2</sub>,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkylaminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**Former Claim 70:**

42. The phosphoinositide analogue of claim 11, wherein:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; and
- (b) a reporter group or conjugand is incorporated in the fatty acyl or inositol residue;

wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**Former Claim 79:**

43. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement and wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**Former Claim 84:**

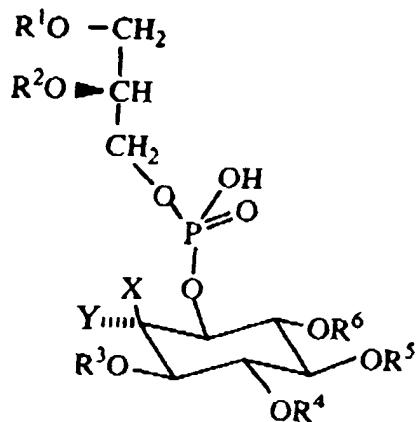
44. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate.

**Former Claim 85:**

45. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate.

**Former Claim 86, Amended as shown:**

46. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glycero ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> is P(=O)(O-protecting group)<sub>2</sub>,

and wherein

- (a) X = OH, and Y = H; or X = H, and Y = OH;

and wherein

- (b) R<sup>1</sup> = RC(=O) or R, R<sup>2</sup> = R'C(=O) or R'  
where R = alkyl, alkenyl, alkynyl, R' =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where R' = alkyl, alkenyl, alkynyl, R =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where R = R', except when R = R' = alkyl;

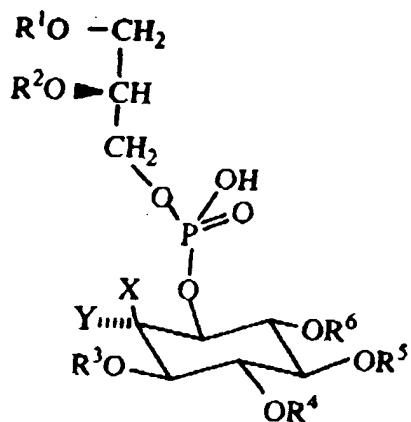
and wherein

- (c) R<sup>3</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,  
(d) R<sup>4</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,  
(e) R<sup>5</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,

- (f)  $R^6 = H, P(=O)(O\text{-protecting group})_2, \omega\text{-aminoalkyl}, \omega\text{-aminoalkenyl}, \omega\text{-sulphydrylalkyl}, \omega\text{-carboxyalkyl}, \omega\text{-(4-azidosalicylamido)-alkyl}, \text{alkylaminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.}$

**Former Claim 87:**

47. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glycero ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of  $R^3, R^4, R^5, R^6$  is  $P(=O)(O\text{-protecting group})_2$ ,

and wherein

- (a)  $X = F, Cl, Br, OC(=O)R, OR, \text{ or } P(=O)(O\text{-protecting group})_2$ , and  $Y = H$ ; or  
 $X = Y = H$ ; or
- (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR, \text{ or } P(=O)(O\text{-protecting group})_2$ , or  
 $X = Y = F \text{ or } (=O)$ ;
- where  $R = \text{alkyl}$ , especially methyl or ethyl, alkenyl, alkynyl,  $\omega\text{-aminoalkyl}$ ,  $N\text{-substituted-}\omega\text{-aminoalkyl}$  or  $N,N\text{-disubstituted-}\omega\text{-aminoalkyl}$ ;

and wherein

- (d)  $R^1 = RC(=O) \text{ or } R, R^2 = R'C(=O) \text{ or } R'$   
where  $R = \text{alkyl}$ , alkenyl, alkynyl,  $R' = \omega\text{-aminoalkyl}$ ,  $\omega\text{-(substitutedamino)-alkyl}$ ,  $\omega\text{-aminoalkenyl}$ ,  $\omega\text{-sulphydrylalkyl}$ ,  $\omega\text{-carboxyalkyl}$ ,  $\omega\text{-(4-azidosalicylamido)-alkyl}$ ,  $\omega\text{-(substitutedamido)-alkyl}$ , alkylaminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' = \text{alkyl}$ , alkenyl, alkynyl,  $R = \omega\text{-aminoalkyl}$ ,  $\omega\text{-(substitutedamino)-alkyl}$ ,  $\omega\text{-aminoalkenyl}$ ,

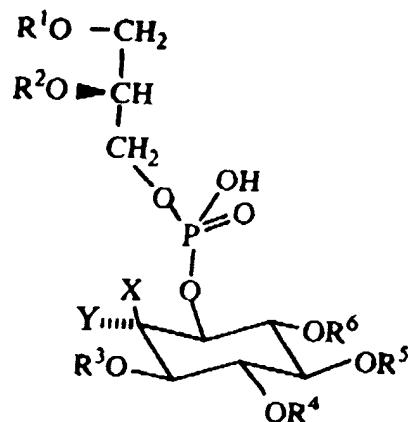
$\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where R = R';

and wherein

- (e)  $R^3$  = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (f)  $R^4$  = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (g)  $R^5$  = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (h)  $R^6$  = H, P(=O)(O-protecting group)<sub>2</sub>,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulfhydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**Former Claim 88:**

48. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement or wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is P(=O)(OH)<sub>2</sub>,

and wherein

- (a) X = F, Cl, Br, OC(=O)R, OR, or OP(=O)(OH)<sub>2</sub>, and Y = H; or  
X = Y = H; or
- (b) X = H, and Y = F, Cl, Br, OC(=O)R, OR, or OP(=O)(OH)<sub>2</sub>; or
- (c) X = Y = F or (=O);  
where R = alkyl, especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

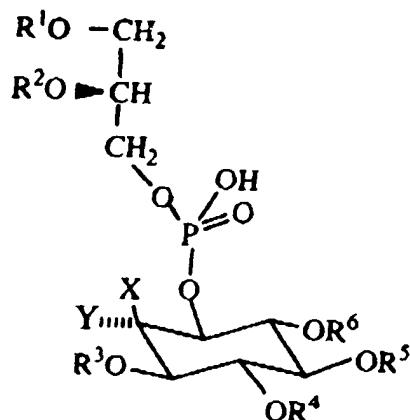
- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R$ ,  $R' =$  alkyl or alkenyl;

and wherein

- (e)  $R^3 = H$ , or  $P(=O)(OH)_2$   
(f)  $R^4 = H$ , or  $P(=O)(OH)_2$   
(g)  $R^5 = H$ , or  $P(=O)(OH)_2$   
(h)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**Former Claim 89, Amended as shown:**

49. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement or wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = OH$ , and  $Y = H$ ; or  $X = H$ , and  $Y = OH$ ;

and wherein

- (b)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R =$  alkyl, alkenyl, alkynyl,  $R' = \omega$ -aminoalkyl,  $\omega$ -(substituted amino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substituted amido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' =$  alkyl, alkenyl,

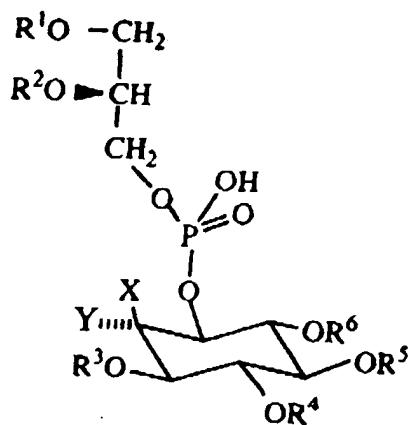
alkynyl, R =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where R = R', except when R = R' = alkyl;

and wherein

- (c)  $R^3 = H$ , or  $P(=O)(OH)_2$
- (d)  $R^4 = H$ , or  $P(=O)(OH)_2$
- (e)  $R^5 = H$ , or  $P(=O)(OH)_2$
- (f)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**Former Claim 90:**

50. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement and a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a) X = F, Cl, Br, OC(=O)R, OR, or OP(=O)(OH)<sub>2</sub>, and Y = H; or  
X = Y = H; or
- (b) X = H, and Y = F, Cl, Br, OC(=O)R, OR, or OP(=O)(OH)<sub>2</sub>; or

- (c)  $X = Y = F$  or  $(=O)$ ;  
 where  $R =$  alkyl, especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
 where  $R =$  alkyl, alkenyl, alkynyl,  $R' = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' =$  alkyl, alkenyl, alkynyl,  $R = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ ;

and wherein

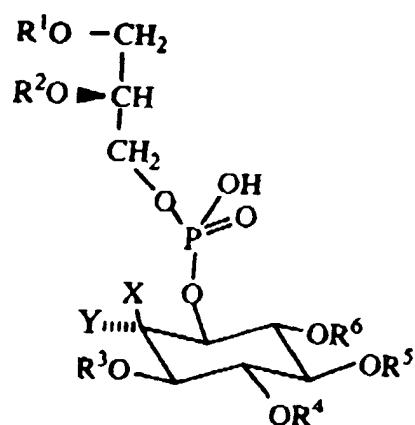
- (e)  $R^3 = H$ , or  $P(=O)(OH)_2$   
 (f)  $R^4 = H$ , or  $P(=O)(OH)_2$   
 (g)  $R^5 = H$ , or  $P(=O)(OH)_2$   
 (h)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

#### Former Claim 91, Amended as shown:

51. Matched pairs of the 2-modified phosphatidylinositol-phosphates of claim 48 and the corresponding phosphatidylinositol-phosphate structure lacking the 2-modification, wherein  $X=OH$  and  $Y=H$ , or  $X=H$  and  $Y=OH$ .

#### New Claim, based upon former Claim 81, Amended:

52. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue has the structure:



wherein at least one of R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> is P(=O)(OH)<sub>2</sub>,

and wherein

- (a) X = OH, and Y = H; or X = H, and Y = OH

and wherein

- (b) R<sup>1</sup> = RC(=O) or R, R<sup>2</sup> = R'C(=O) or R'

where R = alkyl, alkenyl, alkynyl, R' = ω-aminoalkyl, ω-(substitutedamino)-alkyl, ω-aminoalkenyl, ω-sulphydrylalkyl, ω-carboxyalkyl, ω-(4-azidosalicylamido)-alkyl, ω-(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, [alkyl-fluorophor], hydroxylalkyl, or ketoalkyl; or where R' = alkyl, alkenyl, alkynyl, R = ω-aminoalkyl, ω-(substitutedamino)-alkyl, ω-aminoalkenyl, ω-sulphydrylalkyl, ω-carboxyalkyl, ω-(4-azidosalicylamido)-alkyl, ω-(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, hydroxylalkyl, or ketoalkyl;

and wherein

- (c) R<sup>3</sup> = H, or P(=O)(OH)<sub>2</sub>

- (d) R<sup>4</sup> = H, or P(=O)(OH)<sub>2</sub>

- (e) R<sup>5</sup> = H, or P(=O)(OH)<sub>2</sub>

- (f) R<sup>6</sup> = H, P(=O)(OH)<sub>2</sub>, ω-aminoalkyl, ω-aminoalkenyl, ω-sulphydrylalkyl, ω-carboxyalkyl, ω-(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

**New Claim, supported by claim 11:**

53. A phosphoinositide analogue based on di-O-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-O-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein said phosphoinositide analogue incorporates one or more of the following modifying structural features:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; or
- (b) a conjugand suitable for linking to a reporter group, polymer, chromatographic matrix, or gold surface is incorporated in the fattyacyl or inositol residue; wherein said conjugand is selected from the group consisting of ω-aminoalkyl, ω-(substitutedamino)-alkyl, ω-aminoalkenyl, ω-sulphydrylalkyl, ω-carboxyalkyl, hydroxylalkyl and ketoalkyl, and wherein the amino, substitutedamino, sulphydryl, carboxyl, hydroxyl and keto functions are free and unsubstituted, or are covalently linked to a reporter group;

wherein the core structure and absolute stereochemistry of the unmodified di-O-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-O-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**EXHIBIT B**  
**PENDING CLAIMS**  
**CONTINUATION OF SERIAL NO.08/872,222 (NUBI:004--1)**

11. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein said phosphoinositide analogue incorporates one or more of the following modifying structural features:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; or
- (b) a reporter group or conjugand is incorporated in the fatty acyl or inositol residue;

wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

12. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(mono-phosphate) analogue.

13. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(di-phosphate) analogue.

14. The phosphoinositide analogue of claim 13, wherein said phosphoinositide analogue is a PtdIns(4,5)P<sub>2</sub> analogue.

15. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is a phosphoinositide-(poly-phosphate) analogue.

16. The phosphoinositide analogue of claim 11, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement.

17. The phosphoinositide analogue of claim 16, wherein the 2-OH is rendered non-nucleophilic by derivatization.

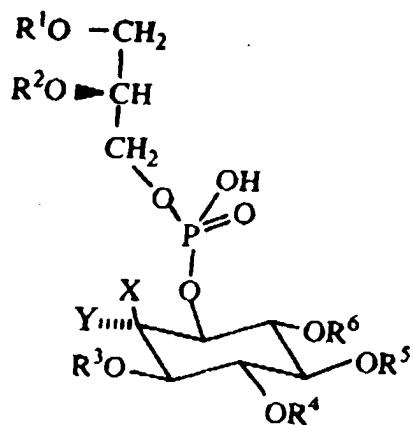
18. The phosphoinositide analogue of claim 17, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is alkyl, substituted alkyl or alkenyl.

19. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form 2-OAc.
20. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is CH<sub>3</sub>.
21. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is  $\omega$ -amino-alkyl.
22. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is N-substituted- $\omega$ -amino-alkyl.
23. The phosphoinositide analogue of claim 18, wherein the 2-OH is rendered non-nucleophilic by derivatization to form a 2-OCOR or 2-OR phosphoinositide analogue, wherein R is N,N-disubstituted- $\omega$ -amino-alkyl.
24. The phosphoinositide analogue of claim 16, wherein the 2-OH is rendered non-nucleophilic by replacement.
25. The phosphoinositide analogue of claim 24, wherein the 2-OH is rendered non-nucleophilic by replacement to form the 2-deoxyhalo or 2-dideoxyhalo phosphoinositide analogue.
26. The phosphoinositide analogue of claim 25, wherein the 2-OH is rendered non-nucleophilic by replacement to form the 2-deoxyfluoro phosphoinositide analogue.
27. The phosphoinositide analogue of claim 11, wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue.
28. The phosphoinositide analogue of claim 27, wherein a reporter group is incorporated.

29. The phosphoinositide analogue of claim 28, wherein the reporter group is a photoaffinity reporter group.
30. The phosphoinositide analogue of claim 28, wherein the reporter group is a fluorescent reporter group.
31. The phosphoinositide analogue of claim 28, wherein the reporter group is a spin probe reporter group.
32. The phosphoinositide analogue of claim 28, wherein the reporter group is a radioactive label reporter group.
33. The phosphoinositide analogue of claim 28, wherein the reporter group is a stable isotope label reporter group.
34. The phosphoinositide analogue of claim 27, wherein a conjugand is incorporated.
35. The phosphoinositide analogue of claim 34, wherein the conjugand is alkyl-C=O,  $\omega$ -NH<sub>2</sub>-alkyl-C=O,  $\omega$ -NH<sub>2</sub>-alkyl,  $\omega$ -thio-(alkyl-C=O) or  $\omega$ -thio-alkyl.
36. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a polymer.
37. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a chromatographic matrix.
38. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a gold surface.
39. The phosphoinositide analogue of claim 34, wherein the conjugand is suitable for linking the phosphoinositide analogue to a reporter group.

40. The phosphoinositide analogue of claim 11, wherein one or both glycerol esters are replaced by ether bonds.

41. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glycero-ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> is P(=O)(O-protecting group)<sub>2</sub>,

and wherein:

- (a) X = F, Cl, Br, OC(=O)R, OR, or P(=O)(O-protecting group)<sub>2</sub>, and Y = H; or X = Y = H; or
- (b) X = H, and Y = F, Cl, Br, OC(=O)R, OR, or P(=O)(O-protecting group)<sub>2</sub>; or
- (c) X = Y = F or (=O);  
where R = alkyl, especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

- (d) R<sup>1</sup> = RC(=O) or R, R<sup>2</sup> = R'C(=O) or R'  
where R, R' = alkyl or alkenyl;

and wherein:

- (e) R<sup>3</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (f) R<sup>4</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (g) R<sup>5</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,

(h)  $R^6$  = H,  $P(=O)(O\text{-protecting group})_2$ ,  $\omega\text{-aminoalkyl}$ ,  $\omega\text{-aminoalkenyl}$ ,  $\omega\text{-sulphydrylalkyl}$ ,  $\omega\text{-carboxyalkyl}$ ,  $\omega\text{-(4-azidosalicylamido)-alkyl}$ , alkylaminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

42. The phosphoinositide analogue of claim 11, wherein:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; and
- (b) a reporter group or conjugand is incorporated in the fatty acyl or inositol residue;

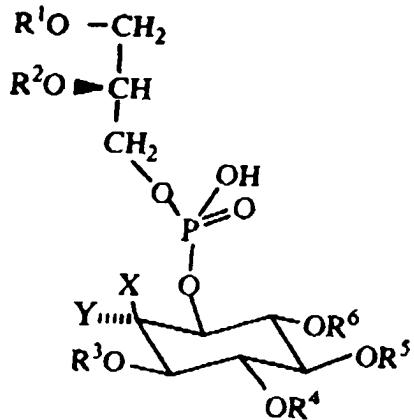
wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

43. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement and wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

44. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate.

45. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue is based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate.

46. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glycero ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup>, R<sup>6</sup> is P(=O)(O-protecting group)<sub>2</sub>,

and wherein

- (a) X = OH, and Y = H; or X = H, and Y = OH;

and wherein

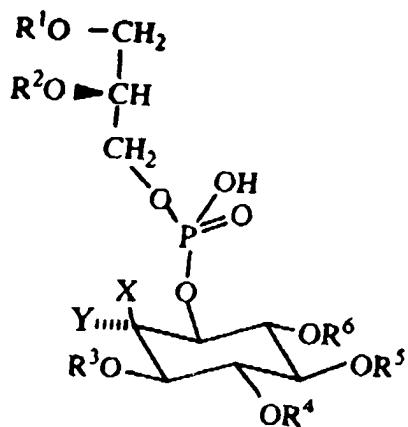
- (b) R<sup>1</sup> = RC(=O) or R, R<sup>2</sup> = R'C(=O) or R'  
where R = alkyl, alkenyl, alkynyl, R' =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where R' = alkyl, alkenyl, alkynyl, R =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where R = R', except when R = R' = alkyl;

and wherein

- (c) R<sup>3</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (d) R<sup>4</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,
- (e) R<sup>5</sup> = H, or P(=O)(O-protecting group)<sub>2</sub>,

- (f)  $R^6 = H, P(=O)(O\text{-protecting group})_2, \omega\text{-aminoalkyl}, \omega\text{-aminoalkenyl}, \omega\text{-sulphydrylalkyl}, \omega\text{-carboxyalkyl}, \omega\text{-(4-azidosalicylamido)\text{-alkyl}}, \text{alkyl}\text{-aminofluorophor, alkyl\text{-amidofluorophor, or alkyl\text{-fluorophor.}}$

47. A selectively *O*-protected phosphoinositide analogue obtained as a phosphodiester intermediate formed by the reaction of a selectively protected *myo*-inositol phosphate or *scyllo*-inositol phosphate and an *sn*-3-phosphatidic acid or glycero ether analogue, wherein the said *O*-protected phosphoinositide analogue has the structure:



wherein at least one of  $R^3, R^4, R^5, R^6$  is  $P(=O)(O\text{-protecting group})_2$ ,

and wherein

- (a)  $X = F, Cl, Br, OC(=O)R, OR,$  or  $P(=O)(O\text{-protecting group})_2$ , and  $Y = H;$  or  
 $X = Y = H;$  or
- (b)  $X = H,$  and  $Y = F, Cl, Br, OC(=O)R, OR,$  or  $P(=O)(O\text{-protecting group})_2,$  or
- (c)  $X = Y = F$  or  $(=O);$   
where  $R = \text{alkyl, especially methyl or ethyl, alkenyl, alkynyl, } \omega\text{-aminoalkyl, } N\text{-substituted-}\omega\text{-aminoalkyl or } N,N\text{-disubstituted-}\omega\text{-aminoalkyl;}$

and wherein

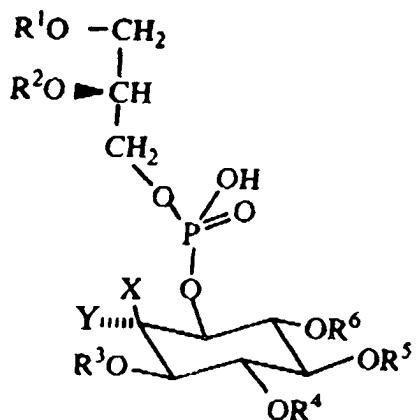
- (d)  $R^1 = RC(=O)$  or  $R,$   $R^2 = R'C(=O)$  or  $R'$   
where  $R = \text{alkyl, alkenyl, alkynyl, } R' = \omega\text{-aminoalkyl, } \omega\text{-(substitutedamino)\text{-alkyl, } }$   
 $\omega\text{-aminoalkenyl, } \omega\text{-sulphydrylalkyl, } \omega\text{-carboxyalkyl, } \omega\text{-(4-azidosalicylamido)\text{-alkyl, } }$   
 $\omega\text{-(substitutedamido)\text{-alkyl, alkyl\text{-aminofluorophor, alkyl\text{-amidofluorophor, }}$   
 $\text{alkyl\text{-fluorophor, hydroxylalkyl, or ketoalkyl; or where } R' = \text{alkyl, alkenyl, }}$   
 $\text{alkynyl, } R = \omega\text{-aminoalkyl, } \omega\text{-(substitutedamino)\text{-alkyl, } }$   
 $\omega\text{-aminoalkenyl, } \omega\text{-sulphydrylalkyl, } \omega\text{-carboxyalkyl, } \omega\text{-(4-azidosalicylamido)\text{-alkyl, }}$

$\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ ;

and wherein

- (e)  $R^3 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (f)  $R^4 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (g)  $R^5 = H$ , or  $P(=O)(O\text{-protecting group})_2$ ,
- (h)  $R^6 = H$ ,  $P(=O)(O\text{-protecting group})_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

48. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement or wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = F, Cl, Br, OC(=O)R, OR, \text{ or } OP(=O)(OH)_2$ , and  $Y = H$ ; or  
 $X = Y = H$ ; or
- (b)  $X = H$ , and  $Y = F, Cl, Br, OC(=O)R, OR, \text{ or } OP(=O)(OH)_2$ ; or
- (c)  $X = Y = F$  or  $(=O)$ ;  
where  $R = \text{alkyl}$ , especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

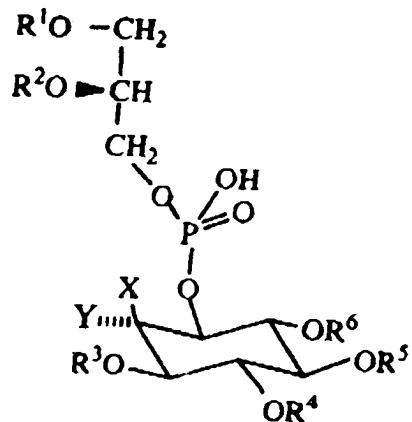
and wherein

- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R, R' = \text{alkyl}$  or alkenyl;

and wherein

- (e)  $R^3 = H$ , or  $P(=O)(OH)_2$
- (f)  $R^4 = H$ , or  $P(=O)(OH)_2$
- (g)  $R^5 = H$ , or  $P(=O)(OH)_2$
- (h)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

49. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement or wherein a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = OH$ , and  $Y = H$ ; or  $X = H$ , and  $Y = OH$ ;

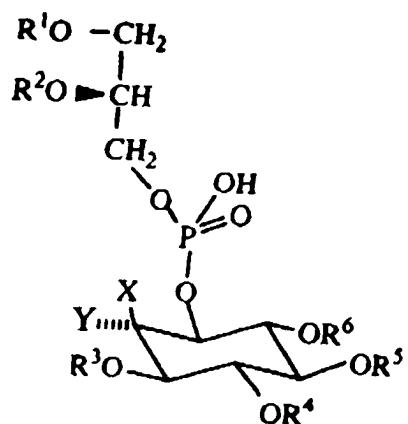
and wherein

- (b)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R$  = alkyl, alkenyl, alkynyl,  $R'$  =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R'$  = alkyl, alkenyl, alkynyl,  $R$  =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ , except when  $R = R' = alkyl$ ;

and wherein

- (c)  $R^3 = H$ , or  $P(=O)(OH)_2$ ,
- (d)  $R^4 = H$ , or  $P(=O)(OH)_2$ ,
- (e)  $R^5 = H$ , or  $P(=O)(OH)_2$ ,
- (f)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

50. A phosphoinositide analogue based on phosphatidylinositolphosphate, wherein the 2-OH is rendered non-nucleophilic by derivatization or replacement and a reporter group or conjugand is incorporated in the fatty acyl or inositol residue; wherein the core structure and absolute stereochemistry of the unmodified phosphatidylinositolphosphate is maintained in said phosphoinositide analogue; and wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = F$ ,  $Cl$ ,  $Br$ ,  $OC(=O)R$ ,  $OR$ , or  $OP(=O)(OH)_2$ , and  $Y = H$ ; or  
 $X = Y = H$ ; or
- (b)  $X = H$ , and  $Y = F$ ,  $Cl$ ,  $Br$ ,  $OC(=O)R$ ,  $OR$ , or  $OP(=O)(OH)_2$ ; or
- (c)  $X = Y = F$  or  $(=O)$ ;  
where  $R =$  alkyl, especially methyl or ethyl, alkenyl, alkynyl,  $\omega$ -aminoalkyl, N-substituted- $\omega$ -aminoalkyl or N,N-disubstituted- $\omega$ -aminoalkyl;

and wherein

- (d)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$   
where  $R =$  alkyl, alkenyl, alkynyl,  $R' = \omega$ -aminoalkyl,  $\omega$ -(substituted amino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-

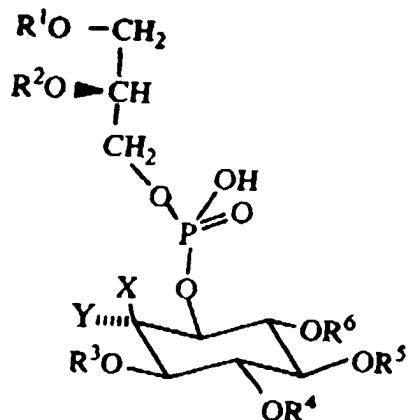
alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R' =$  alkyl, alkenyl, alkynyl,  $R = \omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, alkyl-fluorophor, hydroxylalkyl, or ketoalkyl; or where  $R = R'$ ;

and wherein

- (e)  $R^3 = H$ , or  $P(=O)(OH)_2$
- (f)  $R^4 = H$ , or  $P(=O)(OH)_2$
- (g)  $R^5 = H$ , or  $P(=O)(OH)_2$
- (h)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

51. Matched pairs of the 2-modified phosphatidylinositol-phosphates of claim 48 and the corresponding phosphatidylinositol-phosphate structure lacking the 2-modification, wherein  $X=OH$  and  $Y=H$ , or  $X=H$  and  $Y=OH$ .

52. The phosphoinositide analogue of claim 11, wherein said phosphoinositide analogue has the structure:



wherein at least one of  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$  is  $P(=O)(OH)_2$ ,

and wherein

- (a)  $X = OH$ , and  $Y = H$ ; or  $X = H$ , and  $Y = OH$

and wherein

- (b)  $R^1 = RC(=O)$  or  $R$ ,  $R^2 = R'C(=O)$  or  $R'$

where R = alkyl, alkenyl, alkynyl, R' =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, [alkyl-fluorophor], hydroxylalkyl, or ketoalkyl; or where R' = alkyl, alkenyl, alkynyl, R =  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl,  $\omega$ -(substitutedamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, hydroxylalkyl, or ketoalkyl;

and wherein

- (c)  $R^3 = H$ , or  $P(=O)(OH)_2$
- (d)  $R^4 = H$ , or  $P(=O)(OH)_2$
- (e)  $R^5 = H$ , or  $P(=O)(OH)_2$
- (f)  $R^6 = H$ ,  $P(=O)(OH)_2$ ,  $\omega$ -aminoalkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl,  $\omega$ -(4-azidosalicylamido)-alkyl, alkyl-aminofluorophor, alkyl-amidofluorophor, or alkyl-fluorophor.

53. A phosphoinositide analogue based on di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol having at least one additional hydroxyl group derivatized as a phosphate, wherein said phosphoinositide analogue incorporates one or more of the following modifying structural features:

- (a) the 2-OH is rendered non-nucleophilic by derivatization or replacement; or
- (b) a conjugand suitable for linking to a reporter group, polymer, chromatographic matrix, or gold surface is incorporated in the fattyacyl or inositol residue; wherein said conjugand is selected from the group consisting of  $\omega$ -aminoalkyl,  $\omega$ -(substitutedamino)-alkyl,  $\omega$ -aminoalkenyl,  $\omega$ -sulphydrylalkyl,  $\omega$ -carboxyalkyl, hydroxylalkyl and ketoalkyl, and wherein the amino, substitutedamino, sulphydryl, carboxyl, hydroxyl and keto functions are free and unsubstituted, or are covalently linked to a reporter group;

wherein the core structure and absolute stereochemistry of the unmodified di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*myo*-inositol phosphate or di-*O*-fattyacyl (or alkyl)-*sn*-glycero-3'-phospho-*scyllo*-inositol phosphate is maintained in said phosphoinositide analogue.

**EXHIBIT C**  
**EXPLANATION OF AMENDMENTS**  
**WITH REFERENCE TO SERIAL NO.08/872,222**

The following explanations of the changes in the substitute specification are made with reference to the text of Application Serial No. 08/872,222 as originally filed.

At page 1, line 3 of the text, before "This invention was", the inserted text reads -- The present application claims priority to co-pending application Serial No. 08/872,222, filed June 10, 1997; which claims priority to provisional application Serial No. 60/019,651, filed June 11, 1996. --.

At page 6, line 22 of the text, the deleted text reads "themajor" and the inserted text reads -- the major --.

At page 10, line 23 of the text, in the center of the page, the deleted text reads "methoxybenzyl)-*myo*-inositol" and the inserted text reads -- methoxybenzyl)-*myo/scylo*-inositol --.

At page 11, lines 5, 11, 13, 19, 23 and bridging lines 25 and 26 of the text, each instance of the deleted text reads "-*myo*-inositol" and each instance of the inserted text reads -- -*myo/scylo*-inositol --.

At page 12, line 10 of the text, the deleted text reads "-*myo*-inositol" and the inserted text reads -- -*myo/scylo*-inositol --.

At page 12, lines 15 and 24 of the text, in the center of the page, each instance of the deleted text reads "-*scylo*-inositol" and each instance of the inserted text reads -- -*myo/scylo*-inositol --.

At page 12, line 18 of the text, the deleted text reads "*myo*-inositol" and the inserted text reads -- *myo/scylo*-inositol --.

At page 13, line 2 of the text, the deleted text reads "-*myo*-inositol" and the inserted text reads -- -*myo/scylo*-inositol --.

At page 13, lines 3 and 20 of the text, each instance of the deleted text reads "-*scylo*-inositol" and each instance of the inserted text reads -- -*myo/scylo*-inositol --.

At page 14, lines 6, 8 and 11 of the text, in the center of the page, each instance of the deleted text reads "-*myo*-inositol" and each instance of the inserted text reads -- -*myo/scylo*-inositol --.

At page 14, line 18 of the text, the deleted text reads "1D-3,6-Di-*O*-benzyl-4,5-di-*O*-cyclohexylidene-1-(*p*-methoxybenzyl)-*myo*-inositol" and the inserted text reads -- 1D-3,6-Di-*O*-benzyl-4,5-*O*-cyclohexylidene-1-(*p*-methoxybenzyl)-*myo*-inositol --.

*Exhibit D*

Searching 1999-2000...

**Results of Search in 1999-2000 db for:**  
**"reporter group" OR "reporter molecule": 790 patents.**  
**Hits 1 through 50 out of 790**

 Next 50 hits Print Refine Search | "reporter group" OR "reporter molecule"

- | PAT.<br>NO.  | Title  |
|--------------|--|
| 1 6,114,517  | Methods of modulating tumor necrosis factor .alpha.-induced expression of cell adhesion molecules                    |
| 2 6,114,513  | Thiol-derivatized oligonucleotides   |
| 3 6,114,350  | Cyanine dyes and synthesis methods thereof   |
| 4 6,114,177  | Fluorometric assay for measurement of antioxidant activity   |
| 5 6,114,160  | Compositions and methods for taxol biosynthesis  |
| 6 6,114,117  | Homogeneous diagnostic assay method utilizing simultaneous target and signal amplification                           |
| 7 6,113,904  | Human glycoprotein   |
| 8 6,111,094  | Enhanced antisense modulation of ICAM-1  |
| 9 6,111,085  | Carbamate-derivatized nucleosides and oligonucleosides   |
| 10 6,110,747 | Compounds and methods for modulating tissue permeability   |
| 11 6,110,722 | F.sub.0 ATP synthase subunit   |
| 12 6,110,693 | Methods of assaying receptor activity and constructs useful in such methods  |
| 13 6,110,687 | Detection of antigens via oligonucleotide antibody conjugates  |
| 14 6,110,686 | DNA hybridizing to a human cystatin-like protein (CSTIN)   |
| 15 6,110,682 | Signal enhancement method and kit  |
| 16 6,110,677 | Oligonucleotide modification, signal amplification, and nucleic acid detection by target-catalyzed product formation |
| 17 6,110,675 | Reagents and methods useful for detecting diseases of the prostate   |
| 18 6,110,664 | Antisense inhibition of G-alpha-S1 expression  |
| 19 6,110,630 | Efficient activated cyanine dyes   |
| 20 6,110,507 | Human 3-hydroxyisobutryl-coenzyme a hydrolase  |
| 21 6,107,472 | Receptor-type tyrosine kinase-like molecules   |
| 22 6,107,283 | Cardiac glycosides inhibit proliferation of cells bearing FGF receptors  |

- 23 6,107,092 Antisense modulation of SRA expression  
24 6,107,091 Antisense inhibition of G-alpha-16 expression  
25 6,107,039 Assays using base protected table 1  
26 6,106,844 Immunomodulatory peptides of vespid antigen 5  
27 6,106,732 Integral blood plasma or serum isolation, metering and transport device  
28 6,103,877 Tumor suppressor gene, HIC-1  
29 6,103,874 Human KDEL receptor  
30 6,103,537 Capillary assays involving separation of free and bound species  
31 6,103,497 Human S100 proteins  
32 6,103,483 Molecule involved in binding of sperm to egg surfaces and procedures for use of this molecule to enhance or decrease potential fertility  
33 6,103,479 Miniaturized cell array methods and apparatus for cell-based screening  
34 6,103,477 Rho protein  
35 6,103,474 Hybridization assay signal enhancement  
36 6,103,469 Human phospholipase A2 protein  
37 6,103,217 Polymeric assemblies for sensitive colorimetric assays  
38 6,103,199 Capillary electroflow apparatus and method  
39 6,100,090 Antisense inhibition of PI3K p85 expression  
40 6,100,075 Delta 1-pyrroline-5-carboxylate reductase homolog  
41 6,100,048 Methods and reagents for discovering and using mammalian melanocortin receptor agonists and antagonists to modulate feeding behavior in animals  
42 6,100,040 Methods and compositions for detection of specific nucleotide sequences  
43 6,100,037 Human cyclic nucleotide PDEs  
44 6,100,036 NADH dehydrogenase B17 subunit  
45 6,100,034 Detection of retroviral subtypes based upon envelope specific sequences  
46 6,100,027 Nucleic acid probes and amplification oligonucleotides for *Neisseria* species  
47 6,100,024 Methods and compositions for nucleic acid detection by target extension and probe amplification  
48 6,099,803 Advanced active electronic devices for molecular biological analysis and diagnostics  
49 6,096,725 Methods of using .alpha.Gal oligosaccharides as immune system targeting agents  
50 6,096,722 Antisense modulation of cell adhesion molecule expression and treatment of cell adhesion molecule-associated diseases

Searching 1999-2000...

Results of Search in 1999-2000 db for:  
"reporter group" OR "reporter molecule": 790 patents.  
Hits 51 through 100 out of 790

[Search 50%]

[Search 100%]

[Home To]

[Refine Search] [reporter group" OR "reporter molecule"]

PAT. NO. Title

- 51 6,096,720 Liposomal oligonucleotide compositions
- 52 6,096,543 Antisense inhibition of human mek1 expression
- 53 6,096,502 Substrate for detecting UL9 helicase activity
- 54 6,096,308 Human protein kinase and kinase inhibitors
- 55 6,093,811 Oligonucleotide modulation of cell adhesion
- 56 6,093,809 Telomerase
- 57 6,093,565 Protein phosphatase regulatory subunit
- 58 6,093,538 Nucleic acid probes to ureaplasma
- 59 6,093,537 Double receptor polynucleotide assay method
- 60 6,091,003 Compositions and methods for genetic transformation of pineapple
- 61 6,090,786 Serine proteases, their activity and their synthetic inhibitors
- 62 6,090,631 Methods and compositions for screening for presynaptic calcium channel blockers
- 63 6,090,626 Antisense oligonucleotide modulation of raf gene expression
- 64 6,090,620 Genes and gene products related to Werner's syndrome
- 65 6,090,606 Cleavage agents
- 66 6,090,591 Selective amplification of target polynucleotide sequences
- 67 6,090,589 Nucleic acid amplification with DNA-dependent RNA polymerase activity of RNA replicases
- 68 6,090,578 MTS1 gene
- 69 6,090,577 Disease associated acidic protein
- 70 6,090,564 SnRNP Sm proteins
- 71 6,090,543 Cleavage of nucleic acids
- 72 6,090,390 Diagnostic test for equine arteritis virus mediated disease

9/11/00

- 73 6,090,386 T cell peptides of the CRX JII allergen  
74 6,090,377 Monocyte activating cytokine  
75 6,087,489 Antisense oligonucleotide modulation of human thymidylate synthase expression  
76 6,087,486 Nucleotide sequences encoding vpr receptor protein  
77 6,087,333 Disease associated acidic protein  
78 6,087,178 Method for down regulating CD4 expression in a T cell  
79 6,087,173 Antisense modulation of X-linked inhibitor of apoptosis expression  
80 6,087,172 Ribozymes targeted to human IL-15 mRNA  
81 6,087,125 Polynucleotide encoding a novel human nm23-like protein  
82 6,087,108 RNA editing enzyme  
83 6,084,102 Covalently linked oligonucleotide minor groove binder conjugates  
84 6,084,082 Lactam nucleic acids  
85 6,084,070 Human glutaredoxin beta.  
86 6,083,929 Extended type I chain glycosphingolipids as tumor-associated antigens  
87 6,083,923 Liposomal oligonucleotide compositions for modulating RAS gene expression  
88 6,083,758 Method for screening peptides for metal coordinating properties and fluorescent chemosensors derived therefrom  
89 6,083,750 Adenovirus vectors  
90 6,083,724 Recombinant avian interferon-gamma (IFN-.gamma.)  
91 6,083,706 Inhibitors of leaderless protein export  
92 6,083,704 Human cytochrome b5  
93 6,083,689 Sensitive immunoassays utilizing antibody conjugates with replicable DNA templates  
94 6,080,868 Nitro-substituted non-fluorescent asymmetric cyanine dye compounds  
95 6,080,848 Human brain associated protein  
96 6,080,847 Proteins associated with cell proliferation  
97 6,080,842 Human ATP binding-cassette transport protein  
98 6,080,841 Human induced tumor protein  
99 6,080,723 Human actVA-ORF4-like protein  
100 6,080,558 Polynucleotide encoding human growth regulator protein



US006093538A

**United States Patent [19]**  
**Hogan et al.**

[11] Patent Number: **6,093,538**  
[45] Date of Patent: **Jul. 25, 2000**

**[54] NUCLEIC ACID PROBES TO UREAPLASMA**

[75] Inventors: James J. Hogan, Coronado; Diane L. McAllister, San Diego; Patricia Gordon, Spring Valley; Philip W. Hammond, Tehachapi, all of Calif.

[73] Assignee: Gen-Probe Incorporated, San Diego, Calif.

[21] Appl. No.: **08/109,037**

[22] Filed: **Aug. 18, 1993**

**Related U.S. Application Data**

[63] Continuation-in-part of application No. 07/879,686, May 6, 1992, abandoned.

[51] Int. Cl.<sup>7</sup> ..... C12Q 1/68; C07H 21/04

[52] U.S. Cl. ..... 435/6; 536/24.32; 536/24.33; 536/23.1

[58] Field of Search ..... 435/6; 536/24.32, 536/24.33, 23.1; 935/878; 514/44

**[56] References Cited****U.S. PATENT DOCUMENTS**

4,851,330	7/1989	Kohne .....	435/6
5,030,557	7/1991	Hogan .....	435/6
5,185,439	2/1993	Arnold et al. ....	536/243
5,654,418	8/1997	Sheiness et al. ....	536/24.32
5,843,667	12/1998	Weisburg et al. ....	435/6

**FOREIGN PATENT DOCUMENTS**

0250662	1/1988	European Pat. Off. .	
0281390	9/1988	European Pat. Off. .	
288618	11/1988	European Pat. Off. .	
309230	3/1989	European Pat. Off. .	
313219	4/1989	European Pat. Off. .	
0408295	1/1991	European Pat. Off. .	
8703009	6/1988	WIPO .	

**OTHER PUBLICATIONS**

Robertson et al; *J. Clin Microbiol.* (1993) 31:824-830.

Rogers et al *Proc Natl Acad Sci* (1985) 82:1160-1164.

Van Rappelord et al *Appl Environ Microbiol* (1992) 58:2606-2615.

Hammond et al. 91st General Meeting of the American Soc for Microbiol. (1991) ISSN-0067-2777, Abstract D-17.

Arnold et al *Clin Chem* (1989) 35:1588-1594.

Harsawa et al., "Genomic Characteristics of *Ureaplasma urealyticum*," *Abstract D17, S30-6 UIMS Meeting*, Osaka, Japan (1990).

Robertson et al., "Polymerase Chain Reaction Using 16S rRNA Gene Sequences Distinguishes the Two Biovars of *Ureaplasma urealyticum*," *Journal of Clinical Microbiology* 31:824-830 (1993).

Brunner et al., "Quantitative Studies on the Role of *Ureaplasma urealyticum* in Non-Gonococcal Urethritis and Chronic Prostatitis," *The Yale Journal of Biology and Medicine* 56:545-550 (1983).

Cassell et al., "Role of *Ureaplasma urealyticum* in amnioticitis," *Pediatr. Infect. Dis. t:S247-S252* (1986).

Stagni et al., "Infant Pneumonitis Associated with Cytomegalovirus, *Chlamydia*, *Pneumocystis*, and *Ureaplasma*: A Prospective Study," *Pediatrics* 68:322-329 (1981).

Waites et al., "Chronic *Ureaplasma urealyticum* and *Mycoplasma hominis* Infections of Central Nervous System in Preterm," *The Lancet* 8575:17-21 (1988).

Lee et al., "Molecular Diagnosis of *Ureaplasma urealyticum* Septic Arthritis in a Patient with Hypogammaglobulinemia," *Arthritis and Rheumatism* 35:443-448 (1992).

Roberts et al., "DNA Probes for the Detection of Mycoplasmas in Genital Specimens," *Israel Journal of Medical Sciences* 23:618-620 (1987).

Ohse and Göbel, Analysis of rRNA Operons in *Ureaplasma urealyticum*, *Israel Journal of Medical Sciences* 23:352-356 (1987).

Willoughby et al., "Isolation and Detection of Urease Genes in *Ureaplasma urealyticum*," *Infection and Immunity* 59:2463-2469 (1991).

Deng et al., "Detection of PCR and Differentiation by Restriction Fragment Length Polymorphism of *Acholeplasma*, *Spiroplasma*, *Mycoplasma*, and *Ureaplasma*, Based upon 16S rRNA Genes," *PCR Methods and Applications* 1:202-204 (1992).

Brogan et al., "Development of a DNA probe for *Ureaplasma urealyticum*" *Molecular and Cellular Probes* 6:411-416 (1992).

Weisburg et al., "A Phylogenetic Analysis of the Mycoplasmas: Basis for Their Classification," *Journal of Bacteriology* 171:6455-6467 (1989).

Rogers et al., "Construction of the mycoplasma evolutionary tree from 5S rRNA sequence data," *Proc. Natl. Acad. Sci. USA* 82:1160-1164 (1985).

Southern, "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," *J. Mol. Biol.* 98:503-517 (1975).

Arnold et al., "Assay Formats Involving Acridinium-Ester-Labeled DNA Probes," *Clinical Chemistry* 35:1588-1594 (1989).

Hammond et al., *Abstract D-16, Session 60, American Society for Microbiology Annual Meeting*, (1991).

Gonzales et al., *Abstract D-16, Session 60, American Society for Microbiology Annual Meeting*, (1991).

Stemke and Robertson, "Problems Associated with Serotyping Strains of *Ureaplasma urealyticum*," *Dian. Microbiol. Infect. Dis.* 3:311-320 (1985).

Hammond et al., 91st General Meeting of the American Society for Microbiology (1991) ISSN-0067-2777, Abstract D-17 and Poster Information.

Gonzales et al., 91st General Meeting of the American Society for Microbiology (1991) ISSN-0067-2777, Abstract D-16 and Poster Information.

Gobel et al., "Oligonucleotide probes complementary to variable regions of ribosomal RNA discriminate between Mycoplasma species," *J. General Microbiology* 133:1969-1974 (1987).

Primary Examiner—Carla J. Myers  
Attorney, Agent, or Firm—Brobeck, Phleger & Harrison

[57]

**ABSTRACT**

Hybridization assay probes are described which are able to distinguish *Ureaplasma* and known strains or serotypes of the species *Ureaplasma urealyticum* found in humans from other related organisms.

107 Claims, No Drawings

## NUCLEIC ACID PROBES TO UREAPLASMA

This application is a continuation-in-part of Kacian et al., entitled "Nucleic Acid Sequence Amplification Method, Composition and Kit," U.S. Ser. No. 07/879,686 filed May 6, 1992, now abandoned hereby incorporated by reference herein.

## FIELD OF THE INVENTION

The invention described and claimed herein relates to the design and use of nucleic acid probes capable of detecting organisms of the genus *Ureaplasma*, and known strains or serotypes of the species *Ureaplasma urealyticum*, in test samples, e.g., from urogenital and endocervical specimens, tissue samples, amniotic and other body fluids, and from cultures.

## BACKGROUND OF THE INVENTION

Two single strands of deoxyribo- ("DNA") or ribo- ("RNA") nucleic acid, formed from nucleotides, (including the bases adenine (A), cytosine (C), thymidine (T), guanine (G), uracil (U), or inosine (I)), may hybridize to form a double-stranded structure held together by hydrogen bonds between pairs of complementary bases. Generally, A is hydrogen bonded to T or U, while G or I are hydrogen bonded to C. Along the chain, classical base pairs AT or AU, TA or UA, GC, or CG are present. Additionally, some mismatched base pairs (e.g., AG, GU) may be present.

Bringing together two single strands of nucleic acid containing sufficient contiguous complementary bases, under conditions which will promote their hybridization, results in double-stranded nucleic acid. Under appropriate conditions, DNA/DNA, RNA/DNA, or RNA/RNA hybrids can form.

A probe is generally a single-stranded nucleic acid sequence complementary to some degree to a nucleic acid sequence sought to be detected ("target sequence"). A probe may be labeled with a reporter group moiety such as a radioisotope, a fluorescent or chemiluminescent moiety, or with an enzyme or other ligand which can be used for detection. Background descriptions of the use of nucleic acid hybridization to detect particular nucleic acid sequences are given in Kohne, U.S. Pat. No. 4,851,330 issued Jul. 25, 1989, and by Hogan et al., International Patent Application No. PCT/US87/03009, entitled "Nucleic Acid Probes for Detection and/or Quantitation of Non-Viral Organisms," both references hereby incorporated by reference herein. Hogan et al., supra, describe methods for determining the presence of a non-viral organism or a group of non-viral organisms in a sample (e.g., sputum, urine, blood and tissue sections, food, soil and water).

The genera *Ureaplasma* and *Mycoplasma* are prokaryotes and comprise the taxonomic Mollicutes class. Mollicutes lack a bacterial cell wall and have a small genome size. They are considered one of the smallest of the free-living microorganisms. *Ureaplasma* are unique among Mollicutes because of their characteristic ability to metabolize urea. There are fourteen known serotypes of *U. urealyticum* (Stemke and Robertson, *Diagn. Microbiol. Infect. Dis.* 31: 311 (1985)). The fourteen serotypes can be divided into at least two subspecies ("biotypes") based upon restriction fragment length polymorphism ("RFLP") of *U. urealyticum* genomic DNA (Harasawa et al., *Abstract S30-6 UIMS Meeting*, Osaka Japan (1990), and Robertson et al., *J. Clin. Microbiol.* 31: 824 (1993)), or based upon rRNA sequences (Hammond et al., *Abstract D17. Session 60, American Society for Microbiology General Meeting*, (1991)).

*U. urealyticum* is commonly found in the human urogenital tract but has been implicated in a wide spectrum of pathologies. Several studies have implicated *U. urealyticum* as a possible etiologic agent in diseases affecting adult males, fetuses and infants. Brunner et al., *Yale J. Biol. Med.* 56: 545 (1983), identified *U. urealyticum* as the etiologic agent responsible for nongonococcal urethritis (NGU) in approximately 30 percent of adult males tested who had NGU. Cassell et al., *Pediatr. Infect. Dis.* 5: S247 (1986), implicated *U. urealyticum* as a possible cause of chorioamnionitis, which could in turn adversely affect the outcome of pregnancy and the health of neonates. Stagno et al., *Pediatrics* 68: 322 (1981), found *U. urealyticum* in 21% of infants with pneumonia and found the association of *U. urealyticum* with pneumonia to be "statistically significant." Waites et al., *Lancet* 8575: 17 (1988), found *U. urealyticum* in 8 percent of the cerebrospinal fluid specimens taken from a high-risk population of newborn infants (100 predominantly pre-term infants). According to these investigators *U. urealyticum* was the most common organism isolated of those sought. *U. urealyticum* has also been implicated in a number of other pathogenic states including septic arthritis (Lee et al., *Arthritis and Rheumatism* 35: 43 (1992)).

Standard microbiological techniques generally identify *U. urealyticum* by observing the hydrolysis of urea. These techniques usually involve inoculating both a complex broth medium and an agar medium containing urea and other nutrients with a freshly obtained specimen (Brunner et al., supra).

References concerning detection of *Ureaplasma* include the following: Roberts et al., *Israel J. Med. Sci.* 23: 618 (1987), describe the use of whole chromosomal DNA probes for detection of *Ureaplasma* in genital specimens; Ohse and Göbel, *Israel J. Med. Sci.* 23: 352 (1987) describe hybridization of *U. urealyticum* rRNA genes to cloned DNA of the *E. coli* rRNA operon; Göbel and Stanbridge ("Detection of Mycoplasma by DNA Hybridization", EPO application number 86304919.3, publication number 0 250 662) mention biological probes for detecting Mycoplasmas or prokaryotes in general, or specific Mycoplasma and eubacterial species; Gonzales et al. (*American Society for Microbiology Annual Meeting* 1991, *Abstract D-16*) mentions a method to detect *Ureaplasma* using a DNA probe directed to rRNA; Lee et al., supra, and Willoughby et al., *Infection and Immunity* 59: 2463 (1991), describe a procedure for detecting the *U. urealyticum* urease gene utilizing PCR; Deng et al., *PCR Methods and Applications* 1: 202 (1992), suggest that PCR-RFLP techniques should be capable of detecting Mollicutes; Brogan et al., *Molecular and Cellular Probes* 6: 411 (1992), describe the amplification of a 186 base pair genomic *U. urealyticum* DNA fragment; Robertson et al., supra, describe a technique involving the polymerase chain reaction using biotype specific primers to 16S rRNA gene sequences to distinguish the two *U. urealyticum* biotypes.

## SUMMARY OF THE INVENTION

The featured invention discloses and claims novel oligonucleotide probes which are either targeted to a specific *Ureaplasma* nucleic acid target sequence or consist essentially of a specified nucleic acid sequence. The probes function by hybridizing to target *U. urealyticum* rRNA and/or rRNA gene sequences under stringent hybridization assay conditions. Thus, the probes can distinguish the genus *Ureaplasma*, including clinically significant *U. urealyticum* serotypes, from their known closest phylogenetic neighbors (*Mycoplasma*) and from other microorganism inhabitants of the human urogenital tract. Accordingly, the probes may be

used in an assay to detect and/or quantitate Ureaplasma and *U. urealyticum* organisms.

Species of Mycoplasma found in humans include *M. genitalium*, *M. pneumoniae* and *M. hominis*. *M. pneumoniae* appears to be the most closely related Mycoplasma to *U. urealyticum*. *M. genitalium* is very similar in nucleic acid sequence to *M. pneumoniae* and has been isolated from the human genital tract. *M. hominis* is the most commonly isolated Mycoplasma from the genital tract.

Thus, in a first aspect, the invention described herein features hybridization assay probes able to selectively hybridize to a Ureaplasma target nucleic acid sequence. A Ureaplasma target nucleic acid sequence is a nucleic acid sequence present in Ureaplasma, preferably *U. urealyticum* nucleic acid, or a sequence complementary thereto. Preferably, the target nucleic acid sequence is not present in closely related Mycoplasma (e.g., *M. pneumoniae*). Sequences complementary to a target sequence may be generated by target amplification techniques such as polymerase chain reaction (PCR) or transcription mediated amplification (e.g., Kacian and Fultz, entitled "Nucleic Acid Amplification Methods", EPO application number 90307503.4; and Kacian et al., supra entitled "Nucleic Acid Sequence Amplification Method, Composition and Kit".

The featured probes can detect *U. urealyticum* and distinguish the genus Ureaplasma and known strains or serotypes of *U. urealyticum* found in humans from other bacteria including the phylogenetic closely related *M. pneumoniae*.

A hybridization assay probe is comprised of an oligonucleotide having a nucleic acid sequence sufficiently complementary to hybridize, under stringent hybridization assay conditions, to a 5S, 16S, or 23S rRNA, or to the corresponding ribosomal DNA ("rDNA") nucleic acid sequence, or to a nucleic acid sequence complementary thereto, of *U. urealyticum*. Stringent hybridization assay conditions, refer to conditions wherein a specific probe hybridizes with target nucleic acid (e.g., rRNA of Ureaplasma) and not another nucleic acid present in the test sample from either other microorganisms (e.g., *Mycoplasma pneumonia*) or humans. The probes are preferably 10 to 100 nucleotides in length.

By "probe" is meant to exclude naturally occurring nucleic acids. Purified oligonucleotide probes may be produced by techniques known in the art such as chemical synthesis and by *in vitro* or *in vivo* expression from recombinant nucleic acid molecules, e.g., retroviral vectors.

An oligonucleotide is made of nucleotide subunits covalently joined together. The sugar groups of the nucleotide subunits may be ribose, deoxyribose, or modified derivatives thereof such as O-methyl ribose. The nucleotide subunits may be joined by linkages such as phosphodiester linkages, modified linkages, or by non-nucleotide moieties that do not prevent hybridization of the oligonucleotide. Modified linkages include those linkages in which a standard phosphodiester linkage is replaced with a different linkage, such as a phosphorothioate linkage, or methylphosphonate linkage. When used as a hybridization assay probe, the oligonucleotide preferably contains a reporter group such as acridinium ester or a radioisotope to help identify hybridization of the probe to its target sequence.

In a related aspect, the invention described herein features hybridization assay probes able to selectively hybridize to a *U. urealyticum* nucleic acid target sequence present on either biotype 1 or biotype 2. The claimed target sequence is present in only one of the biotypes. Thus, an oligonucleotide probe directed to either biotype 1 or biotype 2 target site can distinguish between the biotypes.

In another related aspect, hybridization assay probes having a specific nucleic acid sequences complementary to rRNA or rDNA of Ureaplasma, are described. The probes are useful for detecting and/or quantitating Ureaplasma which may be present. These probes are complementary to a region of rRNA or rDNA which varies between Ureaplasma and Mycoplasma. Specific probes able to hybridize to Ureaplasma nucleic acid and distinguish Ureaplasma from Mycoplasma, comprise, consist essentially of, or consist of the sequences (written 5' to 3'):

- (SEQ ID NO: 2) ACCTCTCACT ACAGCTACGC G
- (SEQ ID NO: 5) CAITTCCTAT CTTAGCGTTT CTTCCC
- (SEQ ID NO: 8) CGTTAACGAT CTAGATTAA TAC-CAAACCTT ACAAAACCCG
- 15 (SEQ ID NO: 9) CCTACTACAC TCTAGGTTTA CAGTTTTGA TACAGCTAGA
- (SEQ ID NO: 11) GTCAGTGATA GTCCAAGTTG GC
- (SEQ ID NO: 14) CGTTCGAGCC GACATTTAA TAT-GATCG
- 20 (SEQ ID NO: 17) GCGTCGCAAT AGATGTAAA CCTAG
- (SEQ ID NO: 20) CGATTTGCA GCAGTTGTA TTAGCCATTG
- (SEQ ID NO: 22) GCTAATTTCG GCTCTAGAGT GCT-TGACTTC TGTGTTGGG ATG
- 25 (SEQ ID NO: 23) CGGCTCTAGA GTGCTTGACT TCT-TGTGTTCG
- (SEQ ID NO: 26) GGATGGGAAC AGGTATTTC ACTCTGATAT GATCAC
- 30 (SEQ ID NO: 29) CAGTAATCTA ATTCTCATTA GACT-GAGTTT CCTCATTG and RNA equivalents thereto (SEQ ID NOs: 31, 34, 37, 40, 43, 46, 49, 52, 55, 58, 61, and 109), oligonucleotides complementary thereto (SEQ ID NOs: 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62 and 110), and RNA equivalents to the oligonucleotides complementary thereto (SEQ ID NOs: 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 111). Preferably, helper probe are used to facilitate the hybridization of the assay probe to its target nucleic acid sequence.
- 35 40 The phrases "consists essentially of" or "consisting essentially of" mean that the probe is provided as an oligonucleotide which hybridizes under stringent hybridization assay conditions to a target nucleic acid sequence of a particular organism and preferably does not hybridize with Mycoplasma described herein. A hybridization probe may be linked to other nucleic acids which do not affect hybridization. Generally, it is preferred that the probe be between 10 and 100 (most preferably between 15 and 50) nucleotides in length. Additionally, the probe may be provided in a vector.
- 45 50 For the listed probes, two sets of stringent hybridization assay conditions were used. One set comprised hybridization at 60° C. for one hour in a solution containing 0.095 M lithium succinate pH 5, 0.31 M lithium lauryl sulfate, 1.5 mM ethylenediaminetetraacetic acid (EDTA), 1.5 mM ethylene glycol bis (beta-amino ethyl ether) N, N, N', N' tetraacetic acid (EGTA). After the one hour, hybrids were separated from unhybridized probe by binding to magnetic amine microspheres in a solution containing 0.76 M sodium borate pH 7.5, 6% Triton at 60° C. for ten minutes and washed once in a solution containing 80 mM sodium borate pH 10.4 at room temperature.
- 55 60 Another set of stringent hybridization assay conditions was comprised of hybridization in 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 60° C. for 15 minutes, followed by the addition of 300 µl of 0.6 M sodium borate pH 8.5, 1% Triton X-100 at 60° C. for 5-7 minutes. Additional sets of

stringent hybridization conditions can be determined based upon techniques known in the art and the present disclosure.

In another aspect, specific probes able to distinguish between different biotypes are described. Specific probes able to hybridize to a nucleic acid sequence present in only one *Ureaplasma* biotype comprise, consist essentially of, or consist of the sequences (written 5' to 3'):

SEQ ID NO. 121: CAACACCAGAC TCGTTCGAGC

SEQ ID NO. 122: CAACACCCGAC CCATTCGG and RNA equivalents thereto (SEQ ID NOS: 126 and 127), oligonucleotides complementary thereto (SEQ ID NOS: 131 and 132), and RNA equivalents to the oligonucleotides complementary thereto (SEQ ID NOS: 136 and 137). Preferably, a helper probe is used to facilitate the hybridization of the assay probe to its target nucleic acid sequence.

In another aspect, specific helper probe oligonucleotide sequences have been determined. Helper probes are used to facilitate the rate of hybridization of a hybridization assay probe to its target nucleic acid as described by Hogan and Milliman, U.S. Pat. No. 5,030,557 entitled "Means and Method for Enhancing Nucleic Acid Hybridization," issued Jul. 9, 1991 and hereby incorporated by reference herein. Helper probes featured herein include: SEQ ID NOS. 1, 3, 4, 6, 7, 8, 9, 10, 12, 13, 15, 16, 18, 19, 21, 24, 25, 26, 27, 28, 30, 123, 124, 125; RNA equivalents thereto, SEQ ID NOS. 37, 40, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 97, 100, 103, 106, 109, 112, 115, 118, 128, 129, 130; oligonucleotides complementary thereto, SEQ ID NOS. 38, 41, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107, 110, 113, 116, 119, 133, 134, 135; and RNA equivalents to the oligonucleotides complementary thereto, SEQ ID Nos. 39, 42, 66, 69, 72, 75, 78, 81, 84, 87, 90, 93, 96, 99, 102, 105, 108, 111, 114, 117, 120, 138, 139, 140.

Some oligonucleotide probes can be used as an assay probe or a helper probe (e.g., SEQ ID Nos. 8, 9, and 26, RNA equivalents thereto, SEQ ID Nos. 37, 40, and 109, oligonucleotides complementary thereto, 38, 41, and 110, and RNA equivalents to the oligonucleotides complementary thereto 39, 42, and 111).

In another related aspect, the invention features compositions comprising a nucleic acid hybrid between a hybridization assay probe and a nucleic acid sequence substantially complementary thereto (probe:target). "Substantially complementary" means there is sufficient complementarity between the nucleic acids such that the hybrid is stable under stringent hybridization conditions. One use of the formed hybrid is to detect the presence of a target sequence. For example, acridinium ester ("AE") present in hybrids is resistant to hydrolysis in alkali solution whereas acridinium ester present in single-stranded nucleic acid is hydrolyzed in alkali solution (Arnold et al., entitled "Homogeneous Protection Assay," EPO application number 88308767.8, publication number 309230, hereby incorporated by reference herein). Thus, binding of AE-labeled probe to target can be detected, after hydrolysis of the unbound AE-labeled probe, by measuring chemiluminescence of acridinium ester remaining in the nucleic acid hybrid.

In other related aspects, methods are described for detecting *Ureaplasma urealyticum* and distinguishing *Ureaplasma urealyticum* from Mycoplasma such as *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma capricolum*, *Mycoplasma lipophilum*, and *Mycoplasma salivarium*; distinguishing between *Ureaplasma urealyticum* biotype 1 and *Ureaplasma urealyticum* biotype 2; and detecting the presence of a *Ureaplasma urealyticum* nucleic acid sequence. These methods can be used on test samples obtained from human specimens.

The probes of this invention offer a rapid, non-subjective method of identifying and quantitating the presence of specific rRNA sequences unique to the genus *Ureaplasma* and all strains of *U. urealyticum* in a test sample.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

We have identified preferred target sequences present in the rRNA or rDNA of *U. urealyticum* and designed specific oligonucleotide probes to these sequences and their complements which can be used to identify *Ureaplasma*. The probes can detect the genus *Ureaplasma* including *U. urealyticum* serotypes and distinguish them from their known and presumably most closely related taxonomic or phylogenetic neighbors. Probes are also described which distinguish *U. urealyticum* biotype 1 and biotype 2. Also described are methods using the featured probes or target sites.

In a preferred embodiment, the nucleic acid hybridization assay probes can distinguish *U. urealyticum* from *M. genitalium*, *M. pneumoniae*, or *M. hominis*. In another preferred embodiment, the nucleic acid hybridization probes can distinguish *U. urealyticum* from *M. orale*, *M. fermentans*, *M. capricolum*, *M. lipophilum*, and *M. salivarium*. These *Mycoplasma* have been isolated from humans.

Prokaryotic organisms (excluding viruses) contain rRNA genes encoding 5S rRNA, 16S rRNA and 23S rRNA. Using methods known to those skilled in the art, partial or full rRNA sequences of *U. urealyticum* and *Mycoplasma* were obtained. These sequences were aligned based on regions of sequence homology. Sequence variations were then identified from the aligned sequences and used as target sequences for hybridization assay probes.

#### Obtaining rRNA Sequences

Sequence information was obtained experimentally and from published information (see, Weisburg et al., *J. Bacteriol.* 171: 6455 (1989); and Rogers et al., *Proc. Natl. Acad. Sci., U.S.A.*, 82: 1160 (1985)). Experimental information was obtained by isolating and sequencing the ribonucleic acid from various organisms using sequence standard techniques known in the art. Nucleic acids were obtained using an oligonucleotide primer complementary to a conserved region of rRNA and extending the primer using reverse transcriptase. Nucleic acid sequences were then derived using the method of dideoxynucleotide chain termination. (e.g., Lane et al., *Proc. Natl. Acad. Sci. U.S.A.*, 82: 6955 (1985)).

#### Probe Design And Hybridization Conditions

To facilitate the identification of nucleic acid sequences to be used as probes, the nucleotide sequences from different organisms were first aligned to maximize homology. Within the rRNA molecule there is a close relationship between secondary structure and function. This imposes restrictions on evolutionary changes in the primary sequence so that the secondary structure is maintained. For example, if a base is changed on one side of a helix, a compensating change is made on the other side to preserve the complementarity (this is referred to as co-variance). This allows two very different sequences to be aligned based on the conserved primary sequence and also on the conserved secondary structure elements. Potential target sequences for the hybridization probes were identified by noting variations in the homology of the aligned sequences.

The sequence evolution at each of the variable regions is mostly divergent. Because of the divergence, more distant

phylogenetic relatives of *U. urealyticum* show greater variability to *U. urealyticum* at the variable region than phylogenetically closer relatives. We observed sufficient variation between *U. urealyticum* and strains of Mycoplasma found in the same sample to design several useful probes and identify preferred target sites.

Selective hybridization of probe to target can be accomplished by choosing the appropriate hybridization assay conditions and proper probe design. The stability of the probe:target nucleic acid hybrid should be chosen to be compatible with the assay and washing conditions so that hybrids will only form between highly complementary sequences. Manipulation of one or more of the different assay conditions determines the exact sensitivity and specificity of a particular probe. The following guidelines are useful for designing probes and determining stringent hybridization assay conditions.

Probes should be designed to have an appropriate melting temperature ( $T_m$ ). The appropriate  $T_m$  can be obtained by varying the probe length and nucleotide composition (percentage of G+C versus A+T). The probe length and nucleotide composition should preferably be chosen to correspond to a  $T_m$  about 2–10° C. higher than the temperature at which the final assay will be performed.

In general, the optimal hybridization temperature for oligonucleotide probes of about 10–50 bases in length is approximately 5° C. below the melting temperature for a given duplex. Incubation at temperatures below the optimum temperature may allow mismatched base sequences to hybridize and can therefore decrease specificity. The longer the probe, the more hydrogen bonding between base pairs and, in general, the higher the  $T_m$ . Increasing the percentage of G and C also increases the  $T_m$  because G-C base pairs exhibit additional hydrogen bonding and therefore greater thermal stability than A-T base pairs.

The preferred method to determine  $T_m$  measures hybridization using a Hybridization Protection Assay (HPA) according to Arnold et al., supra entitled "Homogeneous Protection Assay."  $T_m$  can be measured using HPA in the following manner. A probe:target hybrid is formed in a lithium succinate buffered solution (0.1 M lithium succinate buffer, pH 5.0, 2 mM EDTA, 2 mM EGTA, 10% (w/v) lithium lauryl sulfate) using an excess amount of target. Aliquots of the hybrid are then diluted in the lithium succinate buffered solution and incubated for five minutes at various temperatures starting below that of the anticipated  $T_m$  (typically 55° C.) and increasing in 2–5° C. increments. This solution is then diluted with a mild alkaline borate buffer (0.15 M sodium tetraborate, pH 7.6, 5% (v/v) Triton X-100) and incubated at a lower temperature (for example 50° C.) for ten minutes. Under these conditions the acridinium ester attached to a single-stranded probe is hydrolyzed while the acridinium ester attached to hybridized probe is relatively protected from hydrolysis. Thus, the amount of acridinium ester remaining is proportional to the amount of hybrid and can be measured by the chemiluminescence produced from the acridinium ester upon the addition of hydrogen peroxide followed by alkali. Chemiluminescence can be measured in a luminometer (e.g., the Gen-Probe LEADER I or LEADER 50). The resulting data is plotted as percent of maximum signal (usually from the lowest temperature) versus temperature. The  $T_m$  is defined as the temperature at which 50% of the maximum signal remains. In addition to the method above,  $T_m$  may be determined by isotopic methods well known to those skilled in the art (e.g., Hogan et al., supra).

It should be noted that the  $T_m$  for a given hybrid varies depending on the hybridization solution used. Factors such

as the salt concentration, detergents, and other solutes can affect hybrid stability during thermal denaturation (J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular Cloning*, ch. 11 (2d ed. 1989)). Conditions such as ionic strength and incubation temperature under which a probe will be used to hybridize to target should be taken into account in constructing a probe. Thermal stability of hybrids increases as the ionic strength of the reaction mixture increases. On the other hand, chemical reagents which disrupt hydrogen bonds, such as formamide, urea, dimethyl sulfoxide and alcohols, can greatly reduce the thermal stability of the hybrids.

To ensure specificity of probe for target, it is desirable to have probes which hybridize only under conditions of high stringency. Under conditions of high stringency only highly complementary nucleic acid hybrids will form; hybrids without a sufficient degree of complementarity will not form. Accordingly, the stringency of the assay conditions determines the amount of complementarity needed between two nucleic acid strands to form a hybrid. Stringency is chosen to maximize the difference in stability between the hybrid formed with the target and other nucleic acid sequences.

Proper specificity may be achieved by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by constructing the probe to contain as many destabilizing mismatches to nontarget sequences as possible. Whether a probe sequence is useful to detect only a specific type of organism depends largely on the thermal stability difference between probe:target hybrids versus probe:non-target hybrids. In designing probes, the differences in these  $T_m$  values should be as large as possible (preferably 2° C.–5° C. or more).

The length of the target nucleic acid sequence, and accordingly the length of the probe sequence, can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which yield probes with the desired hybridization characteristics. In other cases, one sequence may be significantly better than another which differs merely by a single base. While it is possible for nucleic acids that are not perfectly complementary to hybridize, the longest stretch of perfectly homologous base sequence will generally determine hybrid stability. Oligonucleotide probes of different lengths and base composition may be used. Preferably, oligonucleotide probes are between 10 to 100 and, more preferably, between 15 to 50 bases in length.

Regions of rRNA known to form strong internal structures inhibitory to hybridization are less preferred target regions. Likewise, probes with extensive self-complementarity should be avoided. As explained above, hybridization is the association of two single strands of complementary nucleic acid to form a hydrogen-bonded double strand. It is implicit that if one of the two strands is wholly or partially involved in an intramolecular or intermolecular hybrid it will be less able to participate in the formation of a new intermolecular probe:target hybrid. In the case of rRNA, the molecule is known to form very stable intramolecular hybrids. By designing a probe so that a substantial portion of the targeted sequence is single-stranded, the rate and extent of hybridization between probe and target may be greatly increased.

An rDNA target occurs naturally in a double-stranded form as does the product of the polymerase chain reaction (PCR). These double-stranded targets are naturally inhibitory to hybridization with a probe and require denaturation prior to hybridization. Appropriate denaturation and hybridization conditions are known in the art (e.g., E. M. Southern, *J. Mol. Biol.* 98: 503 (1975)).

**Probe Synthesis**

Once a presumptive unique target sequence has been identified, a complementary oligonucleotide probe is selected and synthesized. Defined oligonucleotide probes may be produced by any of several well-known methods, including automated solid-phase chemical synthesis using cyanoethylphosphoramidite precursors (Barone et al., *Nucleic Acids Research* 12: 4051 (1984)), and as described in J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular Cloning*, ch. 11 (2d ed. 1989). Following synthesis and purification of a particular oligonucleotide probe, several different procedures may be utilized to determine the acceptability of the probe in terms of size and purity. One such procedure is polyacrylamide gel electrophoresis. Another such procedure is High Pressure Liquid Chromatography ("HPLC"). These procedures are well known to those skilled in the art.

Once synthesized, selected oligonucleotide probes may be labeled with a reporter group by any of several well-known methods (e.g., supra, J. Sambrook et al.). Useful labels include radioisotopes and non-radioactive reporting groups. Isotopic labels include <sup>3</sup>H, <sup>35</sup>S, <sup>32</sup>P, <sup>125</sup>I, <sup>57</sup>Co and <sup>14</sup>C. Isotopic labels can be introduced into the oligonucleotide by techniques known in the art such as nick translation, end labeling, second strand synthesis, the use of reverse transcription, and by chemical methods. When using radio-labeled probes, hybridization can be detected by autoradiography, scintillation counting, or gamma counting. The detection method selected will depend upon the particular radioisotope used for labeling.

Non-isotopic materials can also be used for labeling and may be introduced internally into the nucleic acid sequence or at the end of the nucleic acid sequence. Modified nucleotides may be incorporated enzymatically or chemically. Chemical modifications of the probe may be performed during or after synthesis of the probe, for example, through the use of non-nucleotide linker groups as described by Arnold et al., entitled "Non-Nucleotide Linking Reagents for Nucleotide Probes," EPO application number 88308766.0, publication number 313219, hereby incorporated by reference herein. Non-isotopic labels include fluorescent molecules, chemiluminescent molecules, enzymes, cofactors, enzyme substrates, haptens or other ligands.

Preferably, the probes are labeled with an acridinium ester. Acridinium ester labeling may be performed as described by Arnold et al., U.S. Pat. No. 5,185,439 entitled "Acridinium Ester Labeling and Purification of Nucleotide Probes" issued Feb. 9, 1993 and hereby incorporated by reference herein.

**Helper Probes**

The rate of nucleic acid hybridization of an assay probe with its target nucleic acid is enhanced by the use of "Helper Probes" as disclosed in Hogan and Milliman, U.S. Pat. No. 5,030,557 and hereby incorporated by reference herein. Helper probes are selected to hybridize to nucleic acid sequences located near the region targeted by the assay probe. Hybridization of the helper probe alters the secondary and tertiary structure and thereby renders the targeted area of the nucleic acid more accessible for the detection probe. Helper probes to be used with the assay probes described herein include oligonucleotides having the following nucleotide sequences (written 5' to 3'):

(SEQ ID NO: 1) TCATTGACTT GGTGAGCCAT TACCT-CAC  
 (SEQ ID NO: 3) GCCGTGTCTC AGTCCCATTG TGGCT-GTCT  
 (SEQ ID NO: 4) ATATAAAAGA ACTTTACAAT 65 CTATAAGACC TTTCATCGITC ACGGGC

(SEQ ID NO: 6) GGCACATAGT TAGCCGATAC TTAT-TCAAAT GGTACAGTCA AA  
 (SEQ ID NO: 7) CCTGCGCTCG TTTTACGCC  
 AGTAAATCCG GATAACGC  
 5 (SEQ ID NO: 8) CGTTAACAT CTAGATTAA TAC-CAAACCTT ACAACACCG  
 (SEQ ID NO: 9) CCTACTACAC TCTAGGTTA CAGTTTTGA TACAGCTAGA  
 10 (SEQ ID NO: 10) GCCTTCGCCA CCGGTGTTCT TCCATATATC TA  
 (SEQ ID NO: 12) CTAATCCTAT TTGCTCCCCA CACTTCGAG CCTAACG  
 (SEQ ID NO: 13) TTTACGGTGT GGACTACTAG GGTAT  
 (SEQ ID NO: 15) GCGTAGCTA CAACACCGAC T  
 15 (SEQ ID NO: 16) GTAAGGTTCT ACGTGTATTG TCAAATTAAG CAACATGCTC CACCAC  
 (SEQ ID NO: 18) CGACAAACCAT GCACCACCTG TCATATTGTT AACCTCAAC  
 20 (SEQ ID NO: 19) TAGCACGTTT GCAGCCCTAG ATATAAGGGG CATGATG  
 (SEQ ID NO: 21) CGAATTGCAG CCCTCTATCC GAACTGAGAC TAACTTTTC TG  
 25 (SEQ ID NO: 24) GGAACAGGTA TTTCCACTCT GATATGATCA CTAC,  
 (SEQ ID NO: 25) GCGTAGCGAT GACCTATTIT ACT-TGC  
 (SEQ ID NO: 26) GGATGGGAAC AGGTATTCC ACTCTGATAT GATCAC,  
 (SEQ ID NO: 27) GCGTAGCGAT GACCTATTIT ACT-TGCGCTA TTTT  
 (SEQ ID NO: 28) GAGATCAACG GATTAAGGC TCT-TATCAGC TACCCGTTGC TTATCGCAGA TTAG-CACG  
 (SEQ ID NO: 30) CACTTCACCA GGTATCGCTC TGT-TAAACTA TGAATTCACT TATA  
 (SEQ ID NO: 123) CGACATTAA TGATGATCGT  
 40 (SEQ ID NO: 124) GCCGACATT AATGATGATC GTT-TACGGTG TGGAC,  
 (SEQ ID NO: 125) CCCAGGCACA TCATTTAATG CGTTAGCTA, RNA equivalents thereto, SEQ ID NOs. 37, 40, 64, 67, 70, 73, 76, 79, 82, 85, 88, 91, 94, 97, 100, 103, 106, 109, 112, 115, 118, 128, 129, 130; oligonucleotides complementary thereto, SEQ ID NOs. 38, 41, 65, 68, 71, 74, 77, 80, 83, 86, 89, 92, 95, 98, 101, 104, 107, 110, 113, 116, 119, 133, 134, 135; and RNA equivalents to the oligonucleotides complementary thereto, SEQ ID Nos. 39, 42, 66, 69, 72, 75, 78, 81, 84, 87, 90, 93, 96, 98, 102, 105, 108, 111, 114, 117, 120, 138, 139, 140.  
 Preferably, the following hybridization assay probe and helper probe combinations are used:

	Hybridization probe	Helper probes
SEQ ID NOs:	2	1 and 3
SEQ ID NOs:	5	4 and 6
SEQ ID NOs:	8	7 and 9
SEQ ID NOs:	9	8 and 10
SEQ ID NOs:	11	10 and 12
SEQ ID NOs:	14	13 and 15
SEQ ID NOs:	17	16 and 18
SEQ ID NOs:	20	19 and 21
SEQ ID NOs:	22	24 and 25
SEQ ID NOs:	23	26 and 27

-continued

	Hybridization probe	Helper probes
SEQ ID NOs:	29	28 and 30
SEQ ID NOs:	121	123 and 125
SEQ ID NOs:	122	124 and 125

## EXAMPLES

Examples are provided below to illustrate different aspects and embodiments of the present invention. These examples are not intended in any way to limit the disclosed invention.

Probes specific for Ureaplasma were identified by sequencing with primers complementary to the 16S and 23S rRNAs of *U. urealyticum* T-960 (CX-8), or from published 5S sequences. The nucleic acid sequence from phylogenetically near neighbors, including *M. genitalium*, *M. pneumoniae*, *M. iowae*, *M. muris*, *M. pirum* and *M. gallisepticum*, were used as comparisons with the nucleic sequence from *U. urealyticum* to determine variable regions.

The following hybridization assay probe sequences are featured in the examples described below::

(SEQ ID NO: 2) ACCTCTCACT ACAGCTACGC G  
 (SEQ ID NO: 5) CAITTCCTAT CTTAGCGTTT CTTCCC  
 (SEQ ID NO: 8) CGTTAACGAT CTAGATTAA TAC-  
     CAAACCTT ACAAAACCCG  
 (SEQ ID NO: 9) CCTACTACAC TCTAGGTTTA  
     CAGTTTTGA TACAGCTAGA  
 (SEQ ID NO: 11) GTCACTGATA GTCCAAGTTG GC  
 (SEQ ID NO: 14) CGTTCGAGCC GACATTAAAT GAT-  
     GATCG  
 (SEQ ID NO: 17) GCGTCGCAAT AGATGTCAAA  
     CCTAG  
 (SEQ ID NO: 20) CGAIIITTGCA GCAGTTTGT  
     TTAGGCCATTG  
 (SEQ ID NO: 22) GCTAITTTGCG GCTCTAGAGT GCT-  
     TGACTTC TGTTGCGGG ATG  
 (SEQ ID NO: 23) CGGCTCTAGA GTGCTTGACT TCT-  
     GTGTTCG  
 (SEQ ID NO: 29) CAGTAATCTA ATTCTCAITA GACT-  
     GAGTTT CCTCAITCG  
 (SEQ ID NO: 59) CGAACACAGA AGTCAAGCAC  
     TCTAGAGCGG,  
 (SEQ ID NO: 110) GTGATCATAT CAGAGTGGAA  
     ATACCTGTT CCATCC,  
 (SEQ ID NO: 121) CAACACCGAC TCGTTGAGC, and  
 (SEQ ID NO: 122) CAACACCGAC CCATTCGG.

The probes were synthesized with a non-nucleotide linker as described by Arnold et al. supra, "Non-Nucleotide Linking Reagents For Nucleotide Probes," then labeled with a chemiluminescent acridinium ester as described by Arnold et al., supra, U.S. Pat. No. 5,185,439. The reactivity and specificity of the probes for *U. urealyticum* were demonstrated using a hybridization and separation format (Example 1, Tables 1-4) or a homogeneous assay format (Examples 2 and 3, Tables 5 and 6; Example 4, Tables 7 and 8). These procedures are described by Arnold et al., supra, "Homogeneous Protection Assay"; Arnold et al., "Polycationic Supports and Nucleic Acid Purification, Separation and Hybridization" EPO application number 88301839.2, publication number 0 281 390 (hereby incorporated by reference herein); and Arnold et al., *Clin. Chem.*, 35:1588 (1989) (hereby incorporated by reference herein).

Results are given in relative light units (RLU). Probes were hybridized to a cell lysate or RNA purified according

to J. Sambrook, E. F. Fritsch and T. Maniatis, *Molecular Cloning* (2d ed. 1989). Alternatively, lysates, especially of Mycobacteria, Gram positive organisms, or yeasts, could be obtained utilizing a method described by Murphy et al., 5 "Method for Releasing RNA and DNA from Cells," EPO application number 87303641.2, publication number 288618, hereby incorporated by reference herein. The following examples describe hybridization assay probes targeted to *U. urealyticum* rRNA sequences, or the corresponding gene, and their use in a hybridization assay.

## Example 1

This example illustrates the ability of a mixture containing acridinium ester-labeled probes targeted to Ureaplasma 15 16S rRNA to detect various Ureaplasma strains but not other microorganisms. The mixture contained assay probes having SEQ ID NOs. 2, 5, 8, 9, 11, 14, 17 and 20, and the corresponding unlabeled "Helper Probes" (as described above).

Table 1 presents data using these probes with an excess of RNA released from liquid broth cultures containing 10<sup>6</sup>-10<sup>8</sup> organisms. An equal volume of cell lysate and hybridization solution containing 0.19 M lithium succinate pH 5, 0.62 M lithium lauryl sulfate, 3 mM ethylenediaminetetraacetic acid (EDTA), 3 mM ethylene glycol bis (beta-amino ethyl ether) N, N, N', N' tetraacetic acid (EGTA) were mixed and incubated at 60° C. for one hour. Hybrids were then bound to magnetic amine microspheres (Advanced Magnetics, Inc., Cambridge, Mass.) in a solution containing 0.76 M sodium borate pH 7.5, 6% Triton and washed once in a solution containing 80 mM sodium borate pH 10.4. The chemiluminescence associated with the particles, from the hybridized acridinium ester-labeled probes, was measured in a luminometer equipped with automatic injection of 0.1% hydrogen peroxide in 1 mM nitric acid, followed by injection of a 1N sodium hydroxide solution. RLU from a hybridization reaction containing 1 ng of non-target RNA was subtracted from the values shown. The data in Table 1 show that the probes hybridize to known strains or serotypes of *U. urealyticum* found in humans as well as to *U. cati*, *U. diversum* and *U. gallorale* of animal origin.

Table 2 shows that the probes distinguish Ureaplasma from several closely related Mycoplasma, Acholeplasma, or Spiroplasma species. A net RLU value greater than 300 RLU was considered a positive reaction. An all-bacteria/yeast probe mixture was used as a control to demonstrate the presence of bacterial nucleic acid (data not shown). Hogan et al., supra, entitled "Nucleic Acid Probes for Detection and/or Quantitation of Non-Viral Organisms," gives examples of suitable all-bacteria/yeast probe mixtures. The all-bacteria probe used in the examples described herein is a derivative of all-bacteria probe No. 7 described by Hogan et al., (the all-bacteria probe used in the examples described herein is shifted so that it is four nucleotides shorter on the 5' end but 5 bases longer on the 3' end probe than the Hogan probe No. 7). The yeast probe is a derivative of fungal probe No. 1 described in Hogan et al.

Table 3 shows that the assay probe mixture distinguishes Ureaplasma from members of a panel of urogenital microbes. The all-bacteria/yeast probe mixture was also used as a control in this experiment.

Table 4 shows that the assay probes distinguish Ureaplasma from twenty-seven bacterial genera representing a phylogenetic cross section of microorganisms. Again, the all-bacteria/yeast probe mixture was used as a control in this experiment.

TABLE 1

HYBRIDIZATION OF UREAPLASMA 16S rRNA PROBES WITH UREAPLASMA STRAINS AND SEROTYPES			
ATCC NO.	ORGANISM/STRAIN	SEROTYPE	NET RLU <sup>a</sup>
<i>U. urealyticum</i> strain			
27813	7	1	634,146
27618	T-960(CX8)	8	592,533
27814	23	2	775,013
27816	58	4	758,427
27619	K510(CX4)	—	906,488
27815	27	3	703,288
27817	354	5	474,113
27818	Pi	6	769,951
27819	Co	7	780,741
29557	K71-21	4	876,253
29558	K42-35	4	933,227
29559	K12-19	4/8	892,978
33175	Vancouver	9	576,453
33695	K2	11	875,684
33696	U24	12	863,070
33697	U26	14	677,350
33698	U38	13	749,523
33699	Western	10	862,237
49228	<i>U. cati</i>	—	467,562
43321	<i>U. diversum</i>	—	772,938
43346	<i>U. gallorale</i>	—	1,161,922

<sup>a</sup>Chemiluminescence was measured in a Gen-Probe LEADER I luminometer and data are expressed in net Relative Light Units (signal minus the negative control containing 1 ng non-Ureaplasma rRNA).

TABLE 2

HYBRIDIZATION OF UREAPLASMA 16S rRNA PROBES WITH OTHER MOLICUTES			
ORGANISM	ATCC NO.	EXPERIMENT NO.	PROBE MIX NET RLU
<i>Mycoplasma fermentans</i> <sup>a</sup>	15474	1	15
<i>Mycoplasma gallisepticum</i> <sup>a</sup>	19610	1	30
<i>Mycoplasma genitalium</i> <sup>a</sup>	33530	1	31
<i>Mycoplasma hominis</i> <sup>a</sup>	23114	1	38
<i>Mycoplasma iowae</i> <sup>a</sup>	33552	1	81
<i>Mycoplasma muris</i> <sup>a</sup>	33757	1	17
<i>Mycoplasma pirum</i> <sup>a</sup>	25960	1	26
<i>Mycoplasma pneumoniae</i> <sup>a</sup>	15531	1	62
<i>Spiroplasma mirum</i> <sup>a</sup>	29335	1	105
<i>Spiroplasma</i> sp. MQ-1 <sup>a</sup>	33825	1	66
<i>Acholeplasma laidlawii</i> <sup>a</sup>	29804	2	180
<i>Mycoplasma arthritidis</i> <sup>c</sup>	35943	2	14
<i>Mycoplasma buccale</i> <sup>c</sup>	23636	2	58
<i>Mycoplasma oralis</i> <sup>c</sup>	23714	2	-18
<i>Mycoplasma primatum</i> <sup>c</sup>	15497	2	-29
<i>Mycoplasma salivarium</i> <sup>c</sup>	14277	2	11
<i>Ureaplasma urealyticum</i> <sup>b</sup>	27618	2	938

<sup>a</sup>0.10 ng purified RNA.

<sup>b</sup>0.01 ng purified RNA.

<sup>c</sup>Whole cell lysates from 10<sup>7</sup>–10<sup>8</sup> organisms.

TABLE 3

HYBRIDIZATION OF UREAPLASMA 16S rRNA PROBES WITH UROGENITAL MICROBES				
5	ORGANISM <sup>a</sup>	ATCC NO.	UREAPLASMA 16S PROBES NET RLU	ALL-BACTERIA/YEAST PROBES NET RLU
10	<i>Bacteroides fragilis</i>	23745	43	605,178
	<i>Bacteroides ureolyticus</i>	43605	37	112,716
	<i>Candida albicans</i>	18804	26	13,380
	<i>Chlamydia trachomatis</i>	VR-878	-1	76,109
	<i>Clostridium perfringens</i>	13124	-8	419,044
15	<i>Eikenella corrodens</i>	23834	-13	812,060
	<i>Gardnerella vaginalis</i>	14018	11	55,694
	<i>Haemophilus influenzae</i>	9795	6	1,203,162
	<i>Lactobacillus acidophilus</i>	4356	-10	424,616
	<i>Listeria monocytogenes</i>	35152	-8	33,993
	<i>Mycobacterium smegmatis</i>	14468	1	14,392
20	<i>Neisseria gonorrhoeae</i>	19424	122	147,963
	<i>Pasteurellaceae</i>	27337	-8	290,081
	<i>anaerobius</i>			
	<i>Staphylococcus aureus</i>	12598	29	16,256
	<i>Staphylococcus epidermidis</i>	12228	66	4,519
	<i>Torulopsis glabrata</i>	2001	0	646,442

25 \*Whole cell lysates were tested at a concentration of 10<sup>7</sup> cells per reaction.

TABLE 4

HYBRIDIZATION OF UREAPLASMA 16S rRNA PROBES WITH A PHYLOGENETIC PANEL				
30	ORGANISM <sup>a</sup>	ATCC NO.	UREAPLASMA 16S PROBES NET RLU	ALL-BACTERIA/YEAST PROBES NET RLU
35	<i>Ureaplasma urealyticum</i>	27618	1,170	ND
	<i>Alcaligenes faecalis</i>	8750	-6	751,053
	<i>Bacillus subtilis</i>	6051	14	19,523
40	<i>Campylobacter jejuni</i>	33560	2	1,079,901
	<i>Chromobacterium violaceum</i>	29094	10	1,026,462
	<i>Citrobacter freundii</i>	6750	2	758,996
	<i>Actinomyces pyogenes</i>	19411	12	148,548
	<i>Corynebacterium xerosis</i>	373	38	2,091
	<i>Deinococcus radiodurans</i>	35073	-4	78,908
45	<i>Derdia gummosa</i>	15994	20	753,002
	<i>Enterobacter aerogenes</i>	13048	8	967,109
	<i>Enterobacter cloacae</i>	10699	5	1,078,720
	<i>Enterococcus avium</i>	14025	32	10,594
	<i>Enterococcus faecalis</i>	19433	42	32,000
	<i>Erwinia herbicola</i>	33243	9	821,862
	<i>Escherichia coli</i>	10798	66	959,572
50	<i>Klebsiella pneumoniae</i>	23357	12	1,326,216
	<i>Legionella pneumophila</i>	33152	34	869,560
	<i>Micrococcus luteus</i>	9341	50	6,256
	<i>Plesiomonas shigelloides</i>	14029	17	837,909
	<i>Proteus mirabilis</i>	25933	17	927,223
	<i>Pseudomonas aeruginosa</i>	10145	5	1,285,353
55	<i>Pseudomonas fluorescens</i>	13525	10	1,318,299
	<i>Rhodospirillum rubrum</i>	11170	25	563,898
	<i>Streptococcus agalactiae</i>	13813	21	204,717
	<i>Streptococcus bovis</i>	33317	7	402,823
	<i>Vibrio parahaemolyticus</i>	17802	7	1,138,932
	<i>Yersinia enterocolitica</i>	9610	7	1,136,326

60 \*Whole cell lysates were tested at a concentration of 10<sup>7</sup> cells per reaction. The Ureaplasma sample contained 0.01 ng of *Ureaplasma urealyticum* rRNA.  
ND = not done.

#### Example 2

Hybridization of an acridinium ester-labeled probe, targeted to a 23S rRNA *U. urealyticum* region, to *U. urealyticum*.

*cum* and other bacteria was evaluated. Lysate (L) or purified RNA was hybridized to probe SEQ ID NO. 29 and helper probes SEQ ID NOS. 28 and 30 in 0.05 M lithium succinate pH 5, 0.6 M LiCl, 1% (w/v) lithium lauryl sulfate, 10 mM EDTA, 10 mM EGTA at 60° C. for 15 minutes, followed by addition of 300 µl of 0.6 M sodium borate pH 8.5, 1% Triton X-100 at 60° C. for 5–7 minutes. Samples were read in a luminometer as described in Example 1. The *Ureaplasma* sample contained 1 µg of *U. urealyticum* rRNA.

As shown in Table 5, probes targeted to 23S rRNA *U. urealyticum* readily distinguish *U. urealyticum* from other organisms including *Mycoplasma*. The data in this table is reported in RLU without subtracting background and Negative control values. Values greater than about 20,000 to 30,000 RLU were considered positive results in this assay.

TABLE 5

HYBRIDIZATION OF UREAPLASMA-SPECIFIC 23S rRNA PROBES TO OTHER MOLLIICUTES AND <i>E. COLI</i>		
ORGANISM	ATCC NO.	23S PROBE RLU
<i>Mycoplasma arthritidis</i> (L)	35943	746
<i>Mycoplasma buccale</i> (L)	23636	565
<i>Mycoplasma fermentans</i> (L)	15474	948
<i>Mycoplasma iowae</i> (L)	33552	4,241
<i>Mycoplasma muris</i> (L)	33757	4,346
<i>Mycoplasma pirum</i> (L)	25960	596
<i>Mycoplasma primatum</i> (L)	15497	709
<i>Mycoplasma salivarium</i> (L)	14277	629
<i>Spiroplasma</i> sp. MQ-1 (L)	33825	737
<i>Acholeplasma laidlawii</i>	29804	1,052
<i>Mycoplasma gallisepticum</i>	19610	432
<i>Mycoplasma genitalium</i>	33530	4,503
<i>Mycoplasma hominis</i>	23114	450
<i>Mycoplasma oralis</i>	23714	945
<i>Mycoplasma pneumoniae</i>	15531	4,073
<i>Spiroplasma mirum</i>	29335	431
<i>Escherichia coli</i>	10798	772
<i>Ureaplasma urealyticum</i>	27618	1,307,260

## Example 3

Acridinium ester-labeled probe SEQ ID NOS. 22 or 23 targeted to 5S rRNA was hybridized to an excess of RNA released from cells in the form of cell lysate or purified as described above and assayed as described in Example 2. Probe SEQ ID NO. 22 was hybridized in the presence of helper probes SEQ ID NOS. 24 and 25; probe SEQ ID NO. 23 was hybridized in the presence of helper probes SEQ ID NOS. 26 and 27.

As shown in Table 6, the probes targeted to *Ureaplasma urealyticum* 5S rRNA were able to distinguish this organism from other Mollicutes.

TABLE 6

HYBRIDIZATION OF UREAPLASMA 5S rRNA PROBES TO MOLLIICUTES				
ORGANISM	ATCC NO.	PROBE SEQ ID NO. 22 RLU	PROBE SEQ ID NO. 23 RLU	
<i>Mycoplasma arginini</i>	23838	1,332	3,655	
<i>Mycoplasma arthritidis</i> *	35943	1,382	3,957	
<i>Mycoplasma bovigenitalium</i> *	19852	1,395	4,864	
<i>Mycoplasma bovis</i> *	25523	1,280	4,885	
<i>Mycoplasma buccale</i> *	23636	1,332	5,762	
<i>Mycoplasma californicum</i> *	33461	1,466	6,218	

TABLE 6-continued

HYBRIDIZATION OF UREAPLASMA 5S rRNA PROBES TO MOLLIICUTES				
ORGANISM	ATCC NO.	PROBE SEQ ID NO. 22 RLU	PROBE SEQ ID NO. 23 RLU	
<i>Mycoplasma capricolum</i> *	23205	1,496	5,064	
<i>Mycoplasma faecium</i> *	25293	1,466	6,218	
<i>Mycoplasma fermentans</i> *	15474	2,017	10,572	
<i>Mycoplasma gallisepticum</i>	19610	1,355	5,657	
<i>Mycoplasma genitalium</i>	33530	1,233	4,721	
<i>Mycoplasma muris</i> *	33757	5,640	12,462	
<i>Mycoplasma iowae</i> *	33552	2,537	6,498	
<i>Mycoplasma pirum</i> *	25960	1,674	7,354	
<i>Mycoplasma lipophylum</i> *	27790	1,559	5,103	
<i>Mycoplasma neurolyticum</i> *	19988	1,482	5,861	
<i>Mycoplasma oralis</i>	23714	1,697	4,362	
<i>Mycoplasma pneumoniae</i> *	15531	2,129	7,514	
<i>Mycoplasma primatum</i> *	15497	1,530	4,787	
<i>Mycoplasma salivarium</i> *	23064	1,662	4,676	
<i>Spiroplasma mirum</i> *	29335	2,815	7,227	
<i>Ureaplasma urealyticum</i> *	27815	895,233	676,817	
<i>Ureaplasma urealyticum</i> *	27619	1,679,357	1,449,564	

\*Whole cell lysates were tested at a concentration of  $10^7$  cells per reaction.

## Example 4

This example describes probes which can distinguish biotype 1 from biotype 2. In the course of probe development it was observed that one probe gave signals substantially lower for biotype 1 lysates than biotype 2 lysates. This suggested sequence variability in the probe region. To identify probe sequences targeted to a particular biotype several strains of *Ureaplasma urealyticum* were analyzed. Using the sequence information, biotype specific probes SEQ. ID. NOS. 121 and 122 were synthesized and labeled with acridinium ester. The probes were hybridized to rRNA from 18 strains of *Ureaplasma urealyticum* as described in Example 2 and the data is presented in Table 7. The signal obtained with the all-bacteria/yeast probe mix provides a quantitative indication of the amount of rRNA in each sample. The biotype 1 probe reacted only with biotype 1 strains; the biotype 2 probe reacted only with biotype 2 strains.

A similar experiment was performed to investigate the specificity of the biotype probes against 18 closely related *Mycoplasma* species and two *Spiroplasma* species. Results shown in Table 8 are the net RLU (i.e., the RLU from sample tested minus the RLU from a negative control sample). As seen in Table 8, the biotype-specific *Ureaplasma urealyticum* probes reacted only with their respective specific biotype strains and did not cross-react with any of the other closely related organisms.

TABLE 7

HYBRIDIZATION OF BIOTYPE PROBES				
PROBE, NET RLU				
<i>U. urealyticum</i> ATCC NO.	Biotype	All-Bacteria/Yeast	Biotype 1	Biotype 2
27813	1	141,390	10,830	913
27815	1	95,249	60,145	130
27818	1	87,785	30,091	101
33697	1	83,584	77,891	130
27618	2	120,574	120	117,078

TABLE 7-continued

HYBRIDIZATION OF BIOTYPE PROBES				
<i>U. urealyticum</i> ATCC NO.	Biotype	PROBE, NET RLU		
		All-Bacteria/ Yeast	Biotype 1	Biotype 2
27814	2	142,847	5	128,002
27816	2	112,627	89	148,618
27619	2	159,929	958	180,885
27817	2	69,874	108	69,151
27819	2	101,053	61	146,858
29557	2	113,125	143	128,480
29558	2	133,822	55	104,791
29559	2	92,546	644	150,724
33175	2	60,896	93	95,811
33695	2	122,517	106	143,790
33696	2	115,043	183	134,746
33698	2	112,323	3	125,216
33699	2	98,076	125	127,981

TABLE 8

SPECIFICITY OF BIOTYPE PROBES				
ORGANISM	ATCC NO.	PROBE, NET RLU		
		All-Bacteria/ Yeast	Biotype 1	Biotype 2
<i>M. arginini</i>	23838	33,238	230	-184
<i>M. arthritidis</i>	35943	141,240	82	82
<i>M. bovinum</i>	19852	9,543	17	26
<i>M. bovis</i>	25523	70,824	-111	96
<i>M. buccale</i>	23636	15,210	-143	31
<i>M. californicum</i>	33461	113,936	77	26
<i>M. capricolum</i>	23205	50,103	-97	95
<i>M. faecium</i>	25293	61,263	84	21
<i>M. fermentans</i>	15474	34,324	2	-16
<i>M. gallisepticum</i>	19610	62,053	-119	31
<i>M. genitalium</i>	33530	104,629	215	-5
<i>M. pirum</i>	25960	59,082	106	93
<i>M. neurolyticum</i>	19988	17,383	95	72
<i>M. oralis</i>	23715	29,103	22	113
<i>M. pneumoniae</i>	15531	34,329	-161	-94
<i>M. primatum</i>	15497	40,730	-18	23
<i>M. salivarium</i>	23064	66,612	-80	-60
<i>M. hominis</i>	23114	46,680	-58	36
Sp. minum	29335	53,887	-19	34
Sp. MQ-1	33825	35,178	-51	24
<i>U. urealyticum</i> bio. 1	27815	62,491	42,268	163
<i>U. urealyticum</i> bio. 2	27619	108,404	125	136,790

18  
Example 5

This example illustrates the use of assay probes for Ureaplasma of the same sense as the target nucleic acid to detect the products of target nucleic acid amplification.

5 *Ureaplasma urealyticum* rRNA was amplified by incubation at about 37° C. in 100 µL of a solution comprising 0.3 µM of a promoter-primer (SEQ. ID. No. 141), 50 mM Tris-HCl, pH 7.6, 25 mM KCl, 17.5 mM MgCl<sub>2</sub>, 5 mM dithiothreitol, 2 mM spermidine trihydrochloride, 6.5 mM rATP, 2.5 mM 10 rCTP, 6.5 mM rGTP, 2.5 mM rUTP, 0.2 mM DATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.2 mM dTTP, 600 U MuMLV reverse transcriptase and 400 U T7 RNA polymerase (Kacian et al., supra, entitled "Nucleic Acid Sequence Amplification Method, Composition, and Kit"). The reaction was monitored by removing aliquots at various time points between 15 minutes and 4 hours and assaying for the product using two 5S rRNA probes of the same sense as the target rRNA (SEQ. ID. Nos. 59, 110) and helper probes (SEQ. ID. Nos. 104, 107) using conditions described in Example 2.

20 TABLE 9

Time of	RLU	
	Incubation	1 fmol target
25	15 min	5,389
	30 min	10,360
	60 min	40,622
	120 min	144,851
	180 min	192,618
	240 min	203,393
30		307
		778
		5,588
		13,051
		16,249
		20,745

The data shown in Table 9 demonstrates the ability of assay probes targeted to nucleic acid sequences of the opposite sense as the rRNA of the organism to detect the product from a target amplification procedure. As the amplification time increased, more target sequence was produced resulting in increased signal from probe detection.

The data shown in the various examples described above confirm that the novel probes herein described and claimed are capable of distinguishing Ureaplasma from its known nearest phylogenetic neighbors. The data also demonstrates that probes have been designed which can be used to distinguish Ureaplasma biotypes from each other and from nearest known phylogenetic neighbors. Furthermore, complementary oligonucleotide probes, i.e., those having the same sense as the target, are utilized to detect the products of target amplification procedures now being utilized to increase the detection sensitivity of assays for organisms.

45 Other embodiments are within the following claims.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 141

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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19

20

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(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TCATTGACTT GGTGAGCCAT TACCTCAC

28

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ACCTCTCAGT ACAGCTACGC G

21

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCCGTGTCTC AGTCCCATTG TGGCTGTTCT

30

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATATAAAAGA ACTTTACAAT CTATAAGACC TTCATCGTTC ACGCGGC

47

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CATTTCCAT CTTAGCGTTT CTTCCC

26

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGCACATACT TAGCCGATAC TTATTCAAAT GGTACAGTCA AA

42

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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21

22

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CCTCGCGCTCG TTTTACGCC AGTAAATCCG GATAACGC

38

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CGTTAACCAT CTAGATTTAA TACCAAAACTT ACAAAACCG

39

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CCTACTACAC TCTAGGTTTA CAGTTTTGA TACAGCTAGA

40

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GCCTTCGCCA CCGGTGTTCT TCCATATATC TA

32

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GTCAGTGATA GTCCAAGTTG GC

22

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CTAATCCTAT TTGCTCCCCA CACTTTCGAG CCTAACG

37

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

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TTTACGGTGT GGACTACTAG GGAT

25

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	28
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CGTTCGAGCC GACATTTAAC GATGATCG

28

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	21
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GCGPTAGCTA CAACACCGAC T

21

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	46
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GTAAGGTTCT ACGTGTATTG TCAAATTAAG CAACATGCTC CACCAC

46

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	25
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GCGTCGCAAT AGATGTCAAA CCTAG

25

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	39
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CGACAACCAT GCACCACCTG TCATATTGTT AACCTCAAC

39

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	37
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TAGCACGTTT GCAGCCCTAG ATATAAGGGG CATGATG

37

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25

26

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(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CGATTGGCA GCAGTTGTA TTAGCCATTG

30

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 42  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CGAATTGCAG CCCCTCATCC GAACTGAGAC TAACTTTTC TG

42

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 43  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GCTATTTTCG GCTCTAGAGT GCTTGACTTC TGTGTTGGG ATG

43

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CGGCTCTAGA GTGCTTGACT TCTGTTTCG

30

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GGAACAGGTA TTTCCACTCT GATATGATCA CTAC

34

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GCGTAGCGAT GACCTATTTT ACTTGC

26

6,093,538

27

28

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(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GGATGGGAAC AGGTATTTCG ACTCTGATAT GATCAC

36

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CGTAGCGAT GACCTATTTT ACTTGCGCTA TTTT

34

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GAGATCAACG GATTAAGCC TCTTATCAGC TACCCGTTGC TTATCGCAGA TTAGCAGC

58

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

CAGTAATCTA ATTCTCATTA GACTGAGTTT CCTCATTG

39

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

CACTTCACCA GGTATCGTC TGTTAACTA TGAATTCAATT TATA

44

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ACCUCUCAGU ACAGCUACGC G

21

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-continued

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## (2) INFORMATION FOR SEQ ID NO: 32:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CGCGTAGCTG TACTGAGAGG T

21

## (2) INFORMATION FOR SEQ ID NO: 33:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CCCGUAGCUG UACUGAGAGG U

21

## (2) INFORMATION FOR SEQ ID NO: 34:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CAUUUCUUAU CUUAGCGUUU CUUCCC

26

## (2) INFORMATION FOR SEQ ID NO: 35:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GGGAAGAAC GCTAAGATAG GAAATG

26

## (2) INFORMATION FOR SEQ ID NO: 36:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GGGAAGAAC GCUAAGAUAG GAAAUG

26

## (2) INFORMATION FOR SEQ ID NO: 37:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 39
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CGUUAAGCAU CUAGAUUUA UACCAAACUU ACAAACCCG

39

## (2) INFORMATION FOR SEQ ID NO: 38:

6,093,538

31

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CGGGTTTGTA AGTTTGGTAT TAAATCTAGA TGCTTAACG

39

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CAGGUUUGUA AGUUUUGGUAU UAAAUCUAGA UGGCUUAACG

39

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CCUACUACAC UCUAGGUUUA CAGUUUUUGA UACAGCUAGA

40

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

TCTAGCTGTA TCAAAACTG TAAACCTAGA GTGTAGTAGG

40

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

UCUAGCUGUA UCAAAAACUG UAAACCUAGA GUGUAGUAGG

40

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GUCAGUGUA GUCCAAGUUG GC

22

(2) INFORMATION FOR SEQ ID NO: 44:

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## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	22
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GCCAACTTGG ACTATCACTG AC

22

## (2) INFORMATION FOR SEQ ID NO: 45:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	22
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GCCAACUUGG ACUAUCACUG AC

22

## (2) INFORMATION FOR SEQ ID NO: 46:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	28
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (iii) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

CGUUCGAGCC GACAUUUAAU GAUGAUCG

28

## (2) INFORMATION FOR SEQ ID NO: 47:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	28
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (iii) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

CGATCATCAT TAAATGTCGG CTCGAACG

28

## (2) INFORMATION FOR SEQ ID NO: 48:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	28
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

CGAUCAUCAU UAAAUGUCGG CUCGAACG

28

## (2) INFORMATION FOR SEQ ID NO: 49:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	25
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

GCGUCGCAAU AGAUGUAAA CCUAG

25

## (2) INFORMATION FOR SEQ ID NO: 50:

## (i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

CTAGGTTTGA CATCTATTGC GACGC

25

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

CUAGGUUUGA CAUCUAUUGC GACGC

25

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

CGAUUUUGCA GCAGUUUUGUA UUAGCCAUUG

30

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

CAATGGCTAA TACAAACTGC TGCAAAATCG

30

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

CAAUGGCUAA UACAAACUGC UGCCAAAUCG

30

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 43  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

GCUAUUUUCG GCUCUAGAGU GCUUGACUUC UGUGUUCGGG AUG

43

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 43

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(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

CATCCCGAAC ACAGAAAGTCA AGCACACTAG AGCCGAAAAT AGC

43

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 43  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

CAUCCCGAAC ACAGAAAGUCA AGCACACUAG AGCCGAAAAAU AGC

43

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

CGGCUCUAGA GUGCUUGACU UCUGUGUUUCG

30

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

CGAACACAGA AGTCAAGCAC TCTAGAGCCG

30

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

CGAACACAGA AGUCAAGCAC UCUAGAGCCG

30

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 39  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

CAGUAUCUA AUUCUCAUUA GACUGAGUUU CCUCAUUCG

39

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 39  
(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

CGAATGAGGA AACTCAGTCT AATGAGAAATT AGATTACTG

39

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 39  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

CGAAUGAGGA AACUCAGUCU AAUGAGAAUU AGAUUACUG

39

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

UCAUUGACUU GGUGAGCCAU UACCUAC

28

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

GTGAGGTAAT GGCTCACCAA GTCAATGA

28

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

GUGAGGUAAA GGCUCACCAA GUCAAUGA

28

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

GCCGUGUCUC AGUCCCCAUUG UGGCUGUUCU

30

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single

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(D) TOPOLOGY:	linear	
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 68:		
AGAACAGCCA CAATGGGACT GAGACACGGC		30
(2) INFORMATION FOR SEQ ID NO: 69:		
(i) SEQUENCE CHARACTERISTICS:		
(A) LENGTH:	30	
(B) TYPE:	nucleic acid	
(C) STRANDEDNESS:	single	
(D) TOPOLOGY:	linear	
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 69:		
AGACACCCA CAAUAGGACU GAGACACGGC		30
(2) INFORMATION FOR SEQ ID NO: 70:		
(i) SEQUENCE CHARACTERISTICS:		
(A) LENGTH:	47	
(B) TYPE:	nucleic acid	
(C) STRANDEDNESS:	single	
(D) TOPOLOGY:	linear	
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 70:		
AUAUAAAAGA ACUUUACAAU CUUAAAGACC UUCAUCGUUC ACGCGGC		47
(2) INFORMATION FOR SEQ ID NO: 71:		
(i) SEQUENCE CHARACTERISTICS:		
(A) LENGTH:	47	
(B) TYPE:	nucleic acid	
(C) STRANDEDNESS:	single	
(D) TOPOLOGY:	linear	
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 71:		
GCCCGCTGAA CGATGAAGGT CTTATAGATT GTAAAGTTCT TTTATAT		47
(2) INFORMATION FOR SEQ ID NO: 72:		
(i) SEQUENCE CHARACTERISTICS:		
(A) LENGTH:	47	
(B) TYPE:	nucleic acid	
(C) STRANDEDNESS:	single	
(D) TOPOLOGY:	linear	
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 72:		
GCCGCGUGAA CGAUGAAGGU CUUUAUGAUU GUAAAGUUCU UUUUAU		47
(2) INFORMATION FOR SEQ ID NO: 73:		
(i) SEQUENCE CHARACTERISTICS:		
(A) LENGTH:	42	
(B) TYPE:	nucleic acid	
(C) STRANDEDNESS:	single	
(D) TOPOLOGY:	linear	
(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 73:		
GGCACAUAGU UAGCCGAUAC UUAUUCAAAU GGUACAGUCA AA		42
(2) INFORMATION FOR SEQ ID NO: 74:		
(i) SEQUENCE CHARACTERISTICS:		
(A) LENGTH:	42	
(B) TYPE:	nucleic acid	
(C) STRANDEDNESS:	single	
(D) TOPOLOGY:	linear	

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(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

TTTGACTGTAA CCATTTGAAT AAGTATCGGC TAACTATGTG CC

42

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

UUUGACUGUA CCAUUGAAU AAGUAUCGGC UAACUAUGUG CC

42

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

CCUGCGCUUG UUUUACGCC AGUAAAUCGG GAUAACGC

38

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

GCGTTATCCG GATTACTGG GCGTAAAACG AGCGCAGG

38

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

GCGUUAUCCG GAUUUACUGG GCGUAAAACG AGCGCAGG

38

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

GCCUUCGCCA CCCGUGUUUC UCCAUUAUC UA

32

(2) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

TAGATATATG GAAGAACACC GGTGGCGAAG GC

32

(2) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

UAGAUUAUG GAAGAACACC GGUGGCCAAG GC

32

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

CUAAUCCUAU UUGCUCCCCCA CACUUUCGAG CCUAAGC

37

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

GCTTAGGCTC GAAAGTGTGG GGAGCAAATA GGATTAG

37

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

GCUUAGGCUC GAAAGUGUGG GGAGCAAAUA GGAAUAG

37

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

UUUACGGUGU GGACUACUAG GGUAU

25

(2) INFORMATION FOR SEQ ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

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ATACCCCTAGT AGTCCACACC GTAA

25

(2) INFORMATION FOR SEQ ID NO: 87:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

AUACCUAGU AGUCCACACC GUAAA

25

(2) INFORMATION FOR SEQ ID NO: 88:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

GCGGUAGCUA CAACACCGAC U

21

(2) INFORMATION FOR SEQ ID NO: 89:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

AGTCGGTGTT GTAGCTAACG C

21

(2) INFORMATION FOR SEQ ID NO: 90:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

AGUCGGUGUU GUAGCUAACG C

21

(2) INFORMATION FOR SEQ ID NO: 91:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

GUAAGGUUCU ACGUGUAUUG UCAAAUUAAG CAAACUGCUC CACCAC

46

(2) INFORMATION FOR SEQ ID NO: 92:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

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GTCGTGGAGC ATGTTGCTTA ATTGACAAAT ACACGTAGAA CCTTAC

46

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

GUGGUGGAGC AUGUUGC UUA AUUUGACAAU ACACGUAGAA CCUUAC

46

(2) INFORMATION FOR SEQ ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

CGACAACCAU GCACCACCU UCAUAUUGUU ACCCUAAC

39

(2) INFORMATION FOR SEQ ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

GTTGAGGT TA ACAATATGAC AGGTGGTGCA TGGTTGT CG

39

(2) INFORMATION FOR SEQ ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 96:

GUUGAGGUUA ACAAAUAGAC AGGUGGUGCA UGGUUGUCG

39

(2) INFORMATION FOR SEQ ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

UAGCACGUUU GCAGCCCUAG AUUAAGGGG CAUGAUG

37

(2) INFORMATION FOR SEQ ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

CATCATGCC CTTATATCTA GGGCTGAAA CGTGCTA

37

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51

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(2) INFORMATION FOR SEQ ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

CAUCAUGCCC CUUAUAUCUA GGGCUGCAAA CGUGCUA

37

(2) INFORMATION FOR SEQ ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

CGAAUUGCAG CCCUCUAUCC GAACUGAGAC UAACUUUUUC UG

42

(2) INFORMATION FOR SEQ ID NO: 101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

CAGAAAAAGT TACTCTCAGT TCGGATAGAG GGCTGCAATT CG

42

(2) INFORMATION FOR SEQ ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

CAGAAAAAGU UAGUCUCAGU UCGGAUAGAG GGCUGCAAUU CG

42

(2) INFORMATION FOR SEQ ID NO: 103:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

GGAACAGGUA UUUCCACUCU GAUAUGAUCA CUAC

34

(2) INFORMATION FOR SEQ ID NO: 104:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

GTAGTGATCA TATCAGAGTG GAAATACCTG TTCC

34

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## (2) INFORMATION FOR SEQ ID NO: 105:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

GUAGUGAUCA UAUCAAGAGUG GAAAUAACCUG UUCC

34

## (2) INFORMATION FOR SEQ ID NO: 106:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

GCGUAGCGAU GACCUAUUUU ACUUGC

26

## (2) INFORMATION FOR SEQ ID NO: 107:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

GCAAGTAAAA TAGGTTCATCG CTACGC

26

## (2) INFORMATION FOR SEQ ID NO: 108:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

GCAAGUAAAA UAGGUCAUCG CUACGC

26

## (2) INFORMATION FOR SEQ ID NO: 109:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

GGAUGGGAAC AGGUAUUUC ACUCUGAUAU GAUCAC

36

## (2) INFORMATION FOR SEQ ID NO: 110:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

GTGATCATAT CAGAGTGAA ATACCTGTTCC CATCC

36

-continued

## (2) INFORMATION FOR SEQ ID NO: 111:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

GUGAUCAUAU CAGAGUGGAA AUACCUGUUC CCAUCC

36

## (2) INFORMATION FOR SEQ ID NO: 112:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (iii) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

GGGUAGCGAU GACCUAUUUU ACUUGCGCUA UUUU

34

## (2) INFORMATION FOR SEQ ID NO: 113:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 113:

AAAATAGCGC AAGTAAAATA GGTCAATCGCT ACGC

34

## (2) INFORMATION FOR SEQ ID NO: 114:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 114:

AAAAAUAGCGC AAGUAAAAUA GGUCAUCGCU ACGC

34

## (2) INFORMATION FOR SEQ ID NO: 115:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

GAGAUCAACG GAUAAAAGCC UCUUUAUCAGC UACCCGUUGC UUAUCCGAGA UUAGCACCG

58

## (2) INFORMATION FOR SEQ ID NO: 116:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

CGTGCTAACG TGGGATAAGC AACGGGTAGC TGATAGAGG CTTTAATCCG TTGATCTC

58

## (2) INFORMATION FOR SEQ ID NO: 117:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

CGUGCUAAUC UGGCAUAGC AACGGGUAGC UGAUAAGAGG CUUUAUCCG UUGAUCUC 58

(2) INFORMATION FOR SEQ ID NO: 118:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

CACUUCACCA GGUAUCGCUC UGUUAAAACUA UGAUUUCAUU UAUUA 44

(2) INFORMATION FOR SEQ ID NO: 119:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 119:

TATAAAATGAA TTCATAGTTT AACAGAGCGA TACCTGGTGA AGTG 44

(2) INFORMATION FOR SEQ ID NO: 120:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 120:

UAUAAAUGAA UUCAUAGUUU AACAGAGCGA UACCUGGUGA AGUG 44

(2) INFORMATION FOR SEQ ID NO: 121:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 121:

CAACACCGAC TCGTTCGAGC 20

(2) INFORMATION FOR SEQ ID NO: 122:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

CAACACCGAC CCATTCCGG 18

(2) INFORMATION FOR SEQ ID NO: 123:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

CGACATTTAA TGATGATCGT TTACGGTGTG GAC

33

(2) INFORMATION FOR SEQ ID NO: 124:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 124:

GCCGACATTT AATGATGATC GTTTACGGTG TGGAC

35

(2) INFORMATION FOR SEQ ID NO: 125:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 125:

CCCAAGGCACA TCATTTAATG CGTTAGCTA

29

(2) INFORMATION FOR SEQ ID NO: 126:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 126:

CAACACCGAC UCGUUCGAGC

20

(2) INFORMATION FOR SEQ ID NO: 127:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 127:

CAACACCGAC CCAUUCGG

18

(2) INFORMATION FOR SEQ ID NO: 128:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 128:

CGACAUUUAA UGAUGAUCGU UUACGGUGUG GAC

33

(2) INFORMATION FOR SEQ ID NO: 129:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 35  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 129:

GCCGACAUUU AAUGAUGAUC GUUUACGGUG UGGAC 35

(2) INFORMATION FOR SEQ ID NO: 130:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 29  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 130:

CCCAAGGCACA UCAUUAUAUG CGGUAGCUA 29

(2) INFORMATION FOR SEQ ID NO: 131:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 131:

GCTCGAACGA GTCGGTGTTG 20

(2) INFORMATION FOR SEQ ID NO: 132:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 18  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 132:

CCGAATGGGT CGGTGTTG 18

(2) INFORMATION FOR SEQ ID NO: 133:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 33  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 133:

GTCCACACCG TAAACGATCA TCATTAATG TCG 33

(2) INFORMATION FOR SEQ ID NO: 134:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 35  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 134:

GTCCACACCG TAAACGATCA TCATTAATG TCGGC 35

(2) INFORMATION FOR SEQ ID NO: 135:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 29

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(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 135:

TAGCTAACGC ATTAATGAT GTGCCCTGGG

29

(2) INFORMATION FOR SEQ ID NO: 136:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 136:

GCUCGAACGA GUCGGUGUUG

20

(2) INFORMATION FOR SEQ ID NO: 137:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 137:

CCGAAUGGGU CGGUGUUG

18

(2) INFORMATION FOR SEQ ID NO: 138:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 138:

GUCCACACCG UAAACGAUCA UCAUAAAUG UCG

33

(2) INFORMATION FOR SEQ ID NO: 139:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 35  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 139:

GUCCACACCG UAAACGAUCA UCAUAAAUG UCGGC

35

(2) INFORMATION FOR SEQ ID NO: 140:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 140:

UAGCUAACGC AUUAAAUGAU GUGCCUGGG

29

(2) INFORMATION FOR SEQ ID NO: 141:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 53  
(B) TYPE: nucleic acid

-continued

(C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 141:

AATTTAACAC GACTCACTAT AGGGAGAGCG TAGCGATGAC CTATTTTACT TGC

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- What is claimed is:
1. A hybridization assay probe 10 to 100 nucleotides in length comprising an oligonucleotide sufficiently complementary to a *Ureaplasma urealyticum* target nucleic acid sequence to form a detectable probe:target hybrid with said *Ureaplasma urealyticum* target nucleic acid sequence under stringent hybridization assay conditions, wherein said *Ureaplasma urealyticum* target nucleic acid sequence is selected from the group consisting of
  - SEQ ID NO: 31: ACCUCUCAGU ACAGCUACCG G,  
 SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG U,  
 SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA  
 UACCAAACUU ACAAAACCCG,  
 SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUAU  
 UAAAUCUAGA UGCCUUAACG,  
 SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA  
 CAGUUUUUGA UACAGCUAGA,  
 SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG  
 UAAACCUAGA GUGUAGUAGG,  
 SEQ ID NO: 43: GUCAGUGUA GUCCAAGUUG GC,  
 SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG AC,  
 SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA  
 GACUGAGUUU CCUCAUUCG,  
 SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU  
 AAUGAGAAUU AGAUUACUG,  
 SEQ ID NO: 109: GGAUGGGAAC AGGUAUUUCC  
 ACUCUGAAU AUCAUCAC, and  
 SEQ ID NO: 111: GUGAUCAUAU CAGAGUGGAA  
 AUACCUGUC CCAUCC;
  - wherein under said stringent hybridization assay conditions said hybridization assay probe does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma hominis*.
  2. The hybridization assay probe of claim 1, wherein said hybridization assay probe also does not form said detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium* and *Mycoplasma pneumoniae*.
  3. The hybridization assay probe of claim 1, wherein said hybridization assay probe also does not form said detectable probe:non-target hybrid with nucleic acid from *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma capricolum*, *Mycoplasma lipophilum*, and *Mycoplasma salivarium*.
  4. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 31 and SEQ ID NO: 33.
  5. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 37 and SEQ ID NO: 39.
  6. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 40 and SEQ ID NO: 42.
  7. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is
- 10 selected from the group consisting of SEQ ID NO: 43 and SEQ ID NO: 45.
- 15 8. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 61 and SEQ ID NO: 63.
- 20 9. The hybridization assay probe of claim 2, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 109 and SEQ ID NO: 111.
- 25 10. A hybridization assay probe for detecting *Ureaplasma* under stringent hybridization assay conditions which is 21 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:
- 25 SEQ ID NO: 2: ACCTCTCAGT ACAGCTACGC G,  
 SEQ ID NO: 8: CGTTAACAT CTAGATTAA TAC-  
 CAAACTT ACAAAACCCG,  
 SEQ ID NO: 9: CCTACTACAC TCTAGGTTTA  
 CAGTTTTGA TACAGCTAGA,  
 SEQ ID NO: 11: CTCAGTGTATA GTCCAAGTTG GC,  
 SEQ ID NO: 20: CGATTTGCA GCAGTTGTA  
 TTAGCCATTG,  
 SEQ ID NO: 26: GGATGGGAAC AGGTATTCC  
 ACTCTGATAT GATCAC,  
 SEQ ID NO: 29: CAGTAATCTA ATTCTCAITA GACT-  
 GAGTTT CCTCATTGCG,  
 SEQ ID NO: 31: ACCUCUCAGU ACAGCUACCG G,  
 SEQ ID NO: 32: CGCGTAGCTG TACTGAGAGG T,  
 SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG U,  
 SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA  
 UACCAAACUU ACAAAACCCG,  
 SEQ ID NO: 38: CGGGTTTGTA AGTTTGGTAT  
 TAAATCTAGA TGCTTAACG,  
 SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUAU  
 UAAAUCUAGA UGCCUUAACG,  
 SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA  
 CAGUUUUUGA UACAGCUAGA,  
 SEQ ID NO: 41: TCTAGCTGTA TCAAAAACGT  
 TAAACCTAGA GTGTAGTAGG,  
 SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG  
 UAAACCUAGA GUGUAGUAGG,  
 SEQ ID NO: 43: GUCAGUGUA GUCCAAGUUG GC,  
 SEQ ID NO: 44: GCCAACTTGG ACTATCACTG AC,  
 SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG AC,  
 SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA  
 UUAGCCAUUG,  
 SEQ ID NO: 53: CAATGGCTAA TACAAACTGC  
 TGCAAATCG,  
 SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA  
 GACUGAGUUU CCUCAUUCG,  
 SEQ ID NO: 62: CGAATGAGGAAACTCAGTCTAAT-  
 GAGAATT AGATTAACG,  
 SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU  
 AAUGAGAAUU AGAUUACUG,

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SEQ ID NO: 109: GGAUGGGAAC AGGUAUUUCC  
ACUCUGAAU AU GAUCAC,  
SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA  
ATACCTGTTC CCATCC, and  
SEQ ID NO: 111 GUGAUCAUAU CAGAGUGGAA  
AUACCUGUUC CCAUCC;  
wherein said hybridization assay probe hybridizes to *Ureaplasma urealyticum* nucleic acid to form a detectable probe:target hybrid under stringent hybridization assay conditions, but does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions.

11. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33.

12. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39.

13. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42.

14. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45.

15. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 52, and SEQ ID NO: 53.

16. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 109, SEQ ID NO: 110, and SEQ ID NO: 111.

17. The hybridization assay probe of claim 10, wherein said nucleotide base sequence is selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63.

18. The hybridization assay probe of any one of claims 11, 12, 13, 14, 15, 16, and 17, wherein said hybridization assay probe consists of said nucleotide base sequence and one or more reporter groups.

19. A hybridization assay probe 10 to 100 nucleotides in length comprising an oligonucleotide sufficiently complementary to a *Ureaplasma urealyticum* biotype specific target nucleic acid sequence to form a detectable probe:target hybrid under stringent hybridization assay conditions with either *Ureaplasma urealyticum* biotype 1 nucleic acid or *Ureaplasma urealyticum* biotype 2 nucleic acid, wherein said hybridization assay probe does not form said detectable probe:target hybrid with both *Ureaplasma urealyticum* biotype 1 nucleic acid and *Ureaplasma urealyticum* biotype 2 nucleic acid under said stringent hybridization assay conditions, said biotype specific target nucleic acid sequence being selected from the group consisting of:

SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,  
SEQ ID NO: 127: CAACACCGAC CCAUUCGG,  
SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG,  
and

SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG; and  
wherein said hybridization assay probe does not hybridize to nucleic acid from *Mycoplasma genitalium*,

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*Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions.

20. The probe of claim 19, wherein said biotype specific target nucleic acid sequence is either SEQ ID NO: 126 or SEQ ID NO: 136.

21. The probe of claim 19, wherein said biotype specific target nucleic acid sequence is either SEQ ID NO: 127 or SEQ ID NO: 137.

22. A hybridization assay probe for distinguishing between different *Ureaplasma urealyticum* biotypes which is 20 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 121: CAACACCGAC TCGITCGAGC,  
SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,  
SEQ ID NO: 131: GCTCGAACGAGTCGGTGTG, and  
SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG;  
provided that under stringent hybridization assay conditions said hybridization assay probe hybridizes with *Ureaplasma urealyticum* biotype 2 nucleic acid to form a detectable probe:target hybrid, and said hybridization assay probe does not form said detectable probe:target hybrid with *Ureaplasma urealyticum* biotype 1 nucleic acid under said stringent hybridization assay conditions,

further provided that said hybridization assay probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions.

23. The probe of claim 22, wherein said hybridization assay probe consists of said nucleotide base sequence and one or more reporter groups.

24. A hybridization assay probe for distinguishing between different *Ureaplasma urealyticum* biotypes which is 18 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of

SEQ ID NO: 122: CAACACCGAC CCATTGGG,  
SEQ ID NO: 127: CAACACCGAC CCAUUCGG,  
SEQ ID NO: 132: CCGAATGGGT CGGTGTTG, and  
SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG

provided that under stringent hybridization assay conditions said hybridization assay probe hybridizes with *Ureaplasma urealyticum* biotype 1 nucleic acid to form a detectable probe:target hybrid, and said hybridization assay probe does not form said detectable probe:target hybrid with *Ureaplasma urealyticum* biotype 2 nucleic acid under said stringent hybridization assay conditions,

further provided that said hybridization assay probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions.

25. The probe of claim 24, wherein said hybridization assay probe consists of said nucleotide base sequence and one or more reporter groups.

26. A probe mix comprising:

a) a hybridization assay probe for detecting *Ureaplasma* under stringent hybridization assay conditions which is 21 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 2: ACCTCTCAGT ACAGCTACGC G,

SEQ ID NO: 8: CGTTAACAT CTAGATTAA TAC-  
CAAACCT ACAAACCCG,  
SEQ ID NO: 9: CCTACTACAC TCTAGGTTA  
CAGTTTTGA TACAGCTAGA,  
SEQ ID NO: 11: GTCAGTGATA GTCCAAGTTG 5  
GC,  
SEQ ID NO: 20: CGATTTGCA GCAGTTGTA  
TTAGCCATTG,  
SEQ ID NO: 26: GGATGGGAAC AGGTATTCC  
ACTCTGATAT GATCAC,  
SEQ ID NO: 29: CAGTAATCTA ATTCTCATTA  
GACTGAGTTT CCTCATTG,  
SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC  
G,  
SEQ ID NO: 32: CGCGTAGCTG TACTGAGAGG T, 15  
SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG  
U,  
SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUA  
UACCAAACUU ACAAACCCG,  
SEQ ID NO: 38: CGGTTTGTA AGTTGGTAT 20  
TAAATCTAGA TGCTTAACG,  
SEQ ID NO: 39: CGGGUUUGUA AGUTUGGUAU  
UAAAUCUAGA UGUCAAACG,  
SEQ ID NO: 40: CCUACUACAC UCUAGGUUA  
CAGUUUUUGA UACAGCUAGA, 25  
SEQ ID NO: 41: TCTAGCTGTA TCAAAAAACTG  
TAAACCTAGA GTGTAGTAGG,  
SEQ ID NO: 42: UCUAGCUUGUA UCAAAAACUG  
UAAAACCUAGA GUGUAGUAGG,  
SEQ ID NO: 43: GUCAGUGAUA GUCCAAGUUG 30  
GC,  
SEQ ID NO: 44: GCCAACTTGG ACTATCACTG  
AC,  
SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG  
AC,  
SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA 35  
UUAGCCAUG,  
SEQ ID NO: 53: CAATGGCTAA TACAAACTGCG  
TGCAAAATCG,  
SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA 40  
GACUGAGUU CCUCAUUCG,  
SEQ ID NO: 62: CGAATGAGGA AACTCAGTCT  
AATGAGAATT AGATTACTG,  
SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU  
AAUGAGAAUU AGAUUACUG, 45  
SEQ ID NO: 109: GGAUCGGAAC AGGUAUUUC  
ACUCUGUAU GAUCAC,  
SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA  
ATACCTGTTCCATCC, and  
SEQ ID NO: 111: GUGAUCAUAU CAGAGUGGAA 50  
AUACCUGUUC CCAUCC;

wherein under stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* under said stringent hybridization assay conditions; and

b) a helper probe.

27. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 1: TCATTGACTT GGTGAGCCAT  
TACCTCAC,

SEQ ID NO: 3: GCCGTGTCTC AGTCCCATTG  
TGGCTGTTCT,  
SEQ ID NO: 64: UCAUUGACUU GGUGAGCCAU  
UACCUACAC,  
SEQ ID NO: 65: GTGAGGTAAT GGCTCACCAA  
GTCAATGA,

SEQ ID NO: 66: GUGAGGUAAU GGCUCACCAA  
GUCAAUGA,

SEQ ID NO: 67: GCCGUGUCUC AGUCCCAUUG  
UGGCUGUUCU,

SEQ ID NO: 68: AGAACAGCCA CAATGGACT  
GAGACACGGC, and

SEQ ID NO: 69: AGAACAGCCA CAAUGGGACU  
GAGACACGGC.

28. The probe mix of claim 27, wherein said probe mix is selected from the group consisting of:

(a) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 2 or SEQ ID NO: 31;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 1 or SEQ ID NO: 64; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 3 or SEQ ID NO: 67; and

(b) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 32 or SEQ ID NO: 33;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 65 or SEQ ID NO: 66; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 68 or SEQ ID NO: 69.

29. A probe mix comprising:

a) a hybridization assay probe for detecting *Ureaplasma* under stringent hybridization assay conditions which is up to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36; wherein under stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* under said stringent hybridization assay conditions; and

b) a helper probe comprising a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 4: ATATAAAAGA ACTTTACAAT  
CTATAAGACC TTCATCGTT ACAGCGGC,

SEQ ID NO: 70: AUUAUAAAAGA ACUUUACAAU  
CUUAAGAGACC UUCAUCGUUUC ACAGCGGC,

SEQ ID NO: 71: GCGCGTGAA CGATGAAGGT  
CTTATAGATT GTAAAGTTCT TTATAT, and

SEQ ID NO: 72: GCGCGUGAA CGAUGAAGGU  
CUUUAAGAUU GUAAAGUUCU UUUUAU.

30. The probe mix of claim 29, wherein said probe mix is selected from the group consisting of:

(a) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 5 or SEQ ID NO: 34;

- a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 4 or SEQ ID NO: 70; and  
 a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 6 or SEQ ID NO: 73; 5  
 and  
 (b) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 35 or SEQ ID NO: 36; 10  
   a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 71 or SEQ ID NO: 72; and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 74 or SEQ ID NO: 15  
 75.

31. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 7: CCTCGCGCTCG TTTTACGCC  
 AGTAAATCCG GATAACGC,  
 SEQ ID NO: 9: CCTACTACAC TCTAGGTTA 25  
 CAGTTTTGA TACAGCTAGA,  
 SEQ ID NO: 40: CCUACUACAC UCUAGGUAAA  
 CAGUUUUUGA UACAGCUAGA,  
 SEQ ID NO: 41: TCTAGCTGTA TCAAAAAACTG 30  
 TAAACCTAGA GTGTAGTAGG,  
 SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG  
 UAAACCUAGA GUGUAGUAGG,  
 SEQ ID NO: 76: CCUGCGCUCG UUUUACGCC  
 AGUAAAUCCG GAUAACGC,  
 SEQ ID NO: 77: GCGTTATCCG GATTACTGG GCG- 35  
 TAAAACG AGCGCAGG, and  
 SEQ ID NO: 78: GCGUUAUCCG GAUUUACUGG  
 GCGUAAAACG AGCGCAGG.

32. The probe mix of claim 31, wherein said probe mix is selected from the group consisting of:

- (a) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 8 or SEQ ID NO: 37; 45  
   a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 7 or SEQ ID NO: 76; and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 9 or SEQ ID NO: 40; 50

- (b) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 38 or SEQ ID NO: 39; 55  
   a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 77 or SEQ ID NO: 78; and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 41 or SEQ ID NO: 60  
 42.

33. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 8: CGTTAACCAT CTAGATTAA TAC-  
 CAAACTT ACAAACCCG,  
 SEQ ID NO: 10: GCCTTCGCCA CCGGTGTTCT  
 TCCATATATC TA,  
 SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUA  
 UACCAAACUU ACAAACCCG,  
 SEQ ID NO: 38: CGGGTTTGTA AGTTTGGTAT  
 TAAATCTAGA TGCTTAACG,  
 SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUAU  
 UAAAUCUAGA UGCUUUAACG,  
 SEQ ID NO: 79: GCCUUCGCCA CCGGUGUUC  
 UCCAUAUAUC UA,  
 SEQ ID NO: 80: TAGATATATG GAAGAACACC  
 GGTGGCGAAG GC, and  
 SEQ ID NO: 81: UAGAUUAUAG GAAGAACACC  
 GGUGGCGAAG GC.

34. The probe mix of claim 33, wherein said probe mix is selected from the group consisting of:

- (a) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 9 or SEQ ID NO: 40;  
   a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 8 or SEQ ID NO: 37;  
   and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 10 or SEQ ID NO: 79; and  
 (b) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 41 or SEQ ID NO: 42;  
   a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 38 or SEQ ID NO: 39; and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 80 or SEQ ID NO: 81.

35. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 10: GCCTTCGCCA CCGGTGTTCT  
 TCCATATATC TA,  
 SEQ ID NO: 12: CTAATCCTAT TTGCTCCCCA  
 CACTTTCGAG CCTAACG,  
 SEQ ID NO: 79: GCCUUCGCCA CCGGUGUUC  
 UCCAUAUAUC UA,  
 SEQ ID NO: 80: TAGATATATG GAAGAACACC  
 GGTGGCGAAG GC,  
 SEQ ID NO: 81: UAGAUUAUAG GAAGAACACC  
 GGUGGCGAAG GC,  
 SEQ ID NO: 82: CUAAUCCUAU UUGCUCCCCA  
 CACUUUCGAG CCUAAGC,  
 SEQ ID NO: 83: GCTTAGGCTC GAAAGTGTGG  
 GGAGCAAATA GGATTAG, and  
 SEQ ID NO: 84: GCUUAGGCUC GAAAGUGUGG  
 GGAGCAAUA GGAAUAG.

36. The probe mix of claim 35, wherein said probe mix is selected from the group consisting of:

- (a) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 11 or SEQ ID NO: 43;

- a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 10 or SEQ ID NO: 79; and  
 a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 12 or SEQ ID NO: 82; and  
 (b) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 44 or SEQ ID NO: 45;  
   a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 80 or SEQ ID NO: 81; and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 83 or SEQ ID NO: 84.

37. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 52, SEQ ID NO: 53, and SEQ ID NO: 54; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 19: TAGCACGTTT GCAGCCCTAG ATATAAGGGG CATGATG,  
 SEQ ID NO: 21: CGAATTGCAG CCCTCTATCC 25 GAACTGAGAC TAACCTTTC TG,  
 SEQ ID NO: 97: UAGCACGUUU GCAGCCCUAG AUUAAGGGG CAUGAUG,  
 SEQ ID NO: 98: CATCATGCC CTTATATCTA 30 GGGCTGCAA CGTGCTA,  
 SEQ ID NO: 99: CAUCAUGCCC CUUUAUCUA GGGCUGCAA CGUGCUA,  
 SEQ ID NO: 100: CGAAUUGCAG CCCUCUAUCC 35 GAACUGAGAC UAACUUUUUC UG,  
 SEQ ID NO: 101: CAGAAAAAGT TAGTCTCAGT TCGGATAGAG GGCTGCAATT CG, and,  
 SEQ ID NO: 102: CAGAAAAAGU UAGUCUCAGU UCGGAUAGAG GGCUGCAAU CG.

38. The probe mix of claim 37, said probe mix is 40 selected from the group consisting of:

(a) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 20 or SEQ ID NO: 52; 45

  a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 19 or SEQ ID NO: 97; and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 21 or SEQ ID NO: 100; and

(b) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 53 or SEQ ID NO: 54; 55  
   a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 98 or SEQ ID NO: 99; and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 101 or SEQ ID NO: 102.

39. The probe mix of claim 26, wherein said hybridization assay probe comprises a nucleotide base sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63; and said helper probe comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 28: GAGATCAACG GATTAAAGCC TCT-TATCAGC TACCCGTTGC TTATCGCAGA TTAGCACG,

SEQ ID NO: 30: CACTTCACCA GGTATCGCTC TGT-TAAACTA TGAATTCAIT TATA,

SEQ ID NO: 115: GAGAUCAACG GAUAAAAGCC UCUUAUCAGC UACCCGUUGC UUAUCGCAGA UUAGCACG,

SEQ ID NO: 116: CGTGCTAAC TCAGATAAGC AACGGGTAGC TGATAAGAGG CTTTAATCCG TTGATTC,

SEQ ID NO: 117: CGUGCUAAUC UGGCAUAAGC AACGGGUAGC UGAUAAGAGG CUUUAUCCG UUGAUCUC,

SEQ ID NO: 118: CACUUCACCA GGUAUCGCUC UGUUAAACUA UGAAUUCAUU UAUU,

SEQ ID NO: 119: TATAAATGAA TTCATAGTTT AACAGAGCGA TACCTGGTGA AGTG, and

SEQ ID NO: 120: UAUAAAUGAA UUCAUAGUUU AACAGAGCGA UACCUGGUGA AGUG.

40. The probe mix of claim 39, wherein said probe mix is selected from the group consisting of:

(a) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 29 or SEQ ID NO: 61;  
   a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 28 or SEQ ID NO: 115; and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 30 or SEQ ID NO: 118; and

(a) a probe mix comprising  
   a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 62 or SEQ ID NO: 63;  
   a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 116 or SEQ ID NO: 117; and  
   a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 119 or SEQ ID NO: 120.

41. A probe mix comprising

a hybridization assay probe 18 to 100 nucleotides in length comprising a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 121: CAACACCGAC TCGTTCGAGC,  
 SEQ ID NO: 122: CAACACCGAC CCAITCGG,  
 SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,  
 SEQ ID NO: 127: CAACACCGAC CCAUUCGG,  
 SEQ ID NO: 131: GCTCGAACGA GTCGGTGTG,  
 SEQ ID NO: 132: CCGAATGGGT CGGTGTG,  
 SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG, and

SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG;

provided that said hybridization assay probe forms a detectable probe:target hybrid under stringent hybridization assay conditions with either *Ureaplasma urealyticum* biotype 1 nucleic acid or *Ureaplasma urealyticum* biotype 2 nucleic acid, wherein said hybridization assay probe does not form said detectable probe:target hybrid with both *Ureaplasma urealyticum* biotype 1 nucleic acid and *Ureaplasma urealyticum* biotype 2 nucleic acid under said stringent hybridization assay conditions,

further provided that said hybridization assay probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid under said stringent hybridization assay conditions; and

a helper probe comprising a nucleotide base sequence selected from the group consisting of:  
SEQ ID NO: 123: CGACATTAA TGATGATCGT  
TTACGGTGT GAC,  
SEQ ID NO: 124: GCCGACATT AATGATGATC  
GTTTACGGT GGGAC,  
SEQ ID NO: 125: CCCAGGCACA TCATTTAATG  
CGTTAGCTA,  
SEQ ID NO: 128: CGACAUUUUA UGAUGAUCGU  
UUACGGUGUG GAC,  
SEQ ID NO: 129: GCGCACAUUU AAUGAUGAUC  
GUUUACGGUG UGGAC,  
SEQ ID NO: 130: CCCAGGCACA UCAUUUAAAUG  
CGUUAGCUA,  
SEQ ID NO: 133: GTCCACACCG TAAACGATCA  
TCATTTAAATG TCG,  
SEQ ID NO: 134: GTCCACACCG TAAACGATCA  
TCATTTAAATG TCGGC,  
SEQ ID NO: 135: TAGCTAACGC ATTAAATGAT  
GTGCCTGGG,  
SEQ ID NO: 138: GUCCACACCG UAAACGAUCA  
UCAUUAAAUG UCG,  
SEQ ID NO: 139: GUCCACACCG UAAACGAUCA  
UCAUUAAAUG UCGGC, and  
SEQ ID NO: 140: UAGCUAACGC AUUAAAUGAU  
GUGCCUGGG.

42. The probe mix of claim 41, wherein said probe mix is selected from the group consisting of:

(a) a probe mix comprising  
a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 121 or SEQ ID NO: 126,  
a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 123 or SEQ ID NO: 128; and  
a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 125 or SEQ ID NO: 130; and

(b) a probe mix comprising  
a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 131 or SEQ ID NO: 136;  
a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 133 or SEQ ID NO: 138; and  
a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 135 or SEQ ID NO: 140.

43. A probe mix selected from the group consisting of:

(a) a probe mix comprising  
a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 122 or SEQ ID NO: 127,  
a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 124 or SEQ ID NO: 129; and  
a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 125 or SEQ ID NO: 130; and

(b) a probe mix comprising  
a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 132 or SEQ ID NO: 137;  
a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 134 or SEQ ID NO: 139; and  
a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 135 or SEQ ID NO: 140.

44. A probe mix comprising

a hybridization assay probe for detecting *Ureaplasma urealyticum* under stringent hybridization assay conditions which is 28 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:  
SEQ ID NO: 14: CGITCGAGCC GACATTTAAT  
GATGATCG,  
SEQ ID NO: 46: CGUUCGAGCC GACAUUUAAU  
GAUGAUCG,  
SEQ ID NO: 47: CGATCATCAT TAAATGTCGG  
CTCGAACG, and  
SEQ ID NO: 48: CGAUCAUCAU UAAAUGUCGG  
CUCGAACG;

wherein under said stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* under said stringent hybridization assay conditions; and

b) a helper probe consisting of a nucleotide base sequence selected from the group consisting of:  
SEQ ID NO: 13: TTACGGTGT GGACTACTAG  
GGTAT,

SEQ ID NO: 15: GCGTTAGCTA CAACACCGAC T,  
SEQ ID NO: 85: UUUACGGUGU GGACUACUAG  
GGUAU,  
SEQ ID NO: 86: ATACCCTAGT AGTCCACACC  
GTAAC,  
SEQ ID NO: 87: AUACCCUAGU AGUCCACACC  
GUAAA,  
SEQ ID NO: 88: GCGUUAGCUA CAACACCGAC  
U,  
SEQ ID NO: 89: AGTCGGTGT GTAGCTAACG C,  
and  
SEQ ID NO: 90: AGUCGGUGUU GUAGCUAACG  
C.

45. The probe mix of claim 44, wherein said probe mix is selected from the group consisting of:

(a) a probe mix comprising  
a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 14 or SEQ ID NO: 46;  
a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 13 or SEQ ID NO: 85; and  
a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 15 or SEQ ID NO: 88; and

(b) a probe mix comprising  
a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 47 or SEQ ID NO: 48;  
a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 86 or SEQ ID NO: 87; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 89 or SEQ ID NO: 90.

**46. A probe mix comprising**

a) a hybridization assay probe for detecting *Ureaplasma urealyticum* under stringent hybridization assay conditions which is 24 to 100 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 17: GCGTCGCAAT AGATGTCAAA 10 CCTAG,

SEQ ID NO: 49: GCGUCGCAAU AGAUGUCAAA CCUAG,

SEQ ID NO: 50: CTAGGTTGA CATCTATTGC GACGC, and

SEQ ID NO: 51: CUAGGUUUGA CAUCUAUUGC 15 GACGC;

wherein under said stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with a *Ureaplasma urealyticum* target nucleic acid, but does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* under said stringent hybridization assay conditions; and

b) a helper probe consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 16: GTAAGGTTCT ACGTGTATTG TCAAATTAAAG CAACATGCTC CACCAC,

SEQ ID NO: 18: CGACAACCAT GCACCACCTG 30 TCATATTGTT AACCTAACAC,

SEQ ID NO: 91: GUAAGGUUCU ACUGUGUAUUG UCAAAUUAAG CAACAUGCUC CACCAC,

SEQ ID NO: 92: GTGGTGGAGC ATGTTGCTTA ATTGACAAT ACACGTAGAA CCTTAC, 35

SEQ ID NO: 93: GUGGUGGAGC AUGUUGCUUA AUUUGACAAU ACACGUAGAA CCUUAC,

SEQ ID NO: 94: CGACAACCAU GCACCACCG UCAUAUUGU AACCUAAC,

SEQ ID NO: 95: GTTGAGGTTA ACAATATGAC 40 AGGTGGTGCA TGGTTGTCG, and

SEQ ID NO: 96: GUUGAGGUUA ACAUAUUGAC AGGUGGUGCA UGGUUGUCG.

**47. The probe mix of claim 46, wherein said probe mix is selected from the group consisting of:**

(a) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 17 or SEQ ID NO: 49;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 16 or SEQ ID NO: 91; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 18 or SEQ ID NO: 94; and

(b) a probe mix comprising

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 50 or SEQ ID NO: 51;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 92 or SEQ ID NO: 93; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 95 or SEQ ID NO: 96.

**48. A method for detecting the presence of Ureaplasma in a sample and distinguishing said Ureaplasma from *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis* comprising the steps of:**

a) providing to said sample a hybridization assay probe comprising an oligonucleotide which under stringent hybridization assay conditions hybridizes to a *Ureaplasma urealyticum* target nucleic acid selected from the group consisting of:

SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC G,  
SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG U,

SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUA UACCAAACUU ACAAAACCCG,

SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUAU AAAAUCUAGA UGCUUACAC,

SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA CAGUUUUUGA UACAGCUAGA,

SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG UAAACCUAGA GUGUAGUAGG,

SEQ ID NO: 43: GUCAGUGAUA GUCCAAGUUG GC,

SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG AC,

SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA UUAGCCAUG,

SEQ ID NO: 54: CAAUGGUUA UACAAACUGC UGCAAAUCG,

SEQ ID NO: 55: GCUAUUUUCG GCUCUAGAGU GCUUGACUUC UGUGUUCGGG AUG,

SEQ ID NO: 57: CAUCCCGAAC ACAGAAGUCA AGCACUCUAG AGCCGAAAAU AGC,

SEQ ID NO: 58: CGGCUCUAGA GUGCUUGACU UCUGUUUCG,

SEQ ID NO: 60: CGAACACAGA AGUCAAGCAC UCUAGAGCCG,

SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA GACUGAGUUU CCUCAUUCG,

SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU AAUGAGAAU AGAUUACUG,

SEQ ID NO: 109: GGAUGGGAAC AGGUAUUUC ACUCUGAUAU GAUCAC, and

SEQ ID NO: 111: GUGAUCAUAU CAGAGUGGAA AUACCUGUUC CCAUC;

wherein under said stringent hybridization assay conditions said oligonucleotide hybridizes with said target nucleic acid to form a detectable probe:target hybrid and does not hybridize to form a detectable probe:non-target hybrid with *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* nucleic acid under said stringent hybridization assay conditions; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication that Ureaplasma may be present in said sample.

**49. The method of claim 48, wherein target nucleic acid is selected from the group consisting of SEQ ID NO: 31 and SEQ ID NO: 33.**

**50. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 37 and SEQ ID NO: 39.**

**51. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 40 and SEQ ID NO: 42.**

**52. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 43 and SEQ ID NO: 45.**

53. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 52 and SEQ ID NO: 54.

54. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 55 and SEQ ID NO: 57.

55. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 58 and SEQ ID NO: 60.

56. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 61 and SEQ ID NO: 63.

57. The method of claim 48, wherein said target nucleic acid is selected from the group consisting of SEQ ID NO: 109 and SEQ ID NO: 111.

58. A method for detecting the presence of Ureaplasma in a sample and distinguishing said Ureaplasma from *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis*, comprising the steps of:

a) providing to said sample a hybridization assay probe comprising a detection nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 2: ACCTCTCAGT ACAGCTACGC G,

SEQ ID NO: 8: CGTTAACAT CTAGATTTAA TAC-

CAAACCTT ACAAACCCG,

SEQ ID NO: 9: CCTACTACAC TCTAGGTTTA 25 CAGTTTTGA TACAGCTAGA,

SEQ ID NO: 11: GTCAAGTATA GTCCAAGTTG GC,

SEQ ID NO: 20: CGATTTGCA GCAGTTTGTA 30 TTAGCCATTG,

SEQ ID NO: 22: GCTATTTTCG GCTCTAGAGT GCTTGACTTC TGTGTTGGG ATG,

SEQ ID NO: 23: CGGCTCTAGA GTGCTTGACT TCTGTGTTCG,

SEQ ID NO: 26: GGATGGGAAC AGGTATTTCC 35 ACTCTGATAT GATCAC,

SEQ ID NO: 29: CAGTAATCTA ATTCTCATTA GACTGAGTTT CCTCAATTG,

SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC G,

SEQ ID NO: 32: CGCGTAGCTG TACTGAGAGG T,

SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG U,

SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAA UACCAAACUU ACAAACCCG,

SEQ ID NO: 38: CGGGTTTGTA AGTTTGGTAT TAAATCTAGA TGCTTAACG,

SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUUA UAAAUCUAGA UGGCUUACG,

SEQ ID NO: 40: CCUACUACAC UCUAGGUUUA 50 CAGUUUUUGA UACAGCUAGA,

SEQ ID NO: 41: TCTAGCTGTA TCAAAAACGT TAAACCTAGA GTGTAGTAGG,

SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG UAAACCUAGA GUGUAGUAGG,

SEQ ID NO: 43: GUCAGUGUA GUCCAAGUUG GC,

SEQ ID NO: 44: GCCAACTTGG ACTATCACTG AC,

SEQ ID NO: 45: GCCAACUUGG ACUAUCACUG 60 AC,

SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA UUAGCCAUUG,

SEQ ID NO: 53: CAATGGCTAA TACAAACTGC TGCAAAATCG,

SEQ ID NO: 54: CAAUGGCUAA UACAAACUGC UGCAAAUCG,

SEQ ID NO: 55: GCUAUUUUCG GCUCUAGAGU GCUJUGACUUC UGUGUUUCGGG AUG,

SEQ ID NO: 56: CATCCCGAAC ACAGAAGTCA AGCACTCTAG AGCCGAAAAT AGC,

SEQ ID NO: 57: CAUCCCGAAC ACAGAAGUCA AGCACUCUAG AGCCGAAAAT AGC,

SEQ ID NO: 58: CGGCUCUAGA GUGCUUGACU UCUGUGUUCG,

SEQ ID NO: 59: CGAACACAGA AGTCAAGCAC TCTAGAGCCG,

SEQ ID NO: 60: CGAACACAGA AGUCAAGCAC UCUAGAGCCG,

SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA GACUGAGAUU CCUCAUUCG,

SEQ ID NO: 62: CGAATGAGGA AACTCAGTCT AATGAGAAATT AGATTACTG,

SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU AAUGAGAAAU AGAAUACUG,

SEQ ID NO: 109: GGAUGGGAAC AGGUAUUUC ACUCUGAUU GAUCAC,

SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA ATACCTGTTTCCATCC, and

SEQ ID NO: 111: GUGAUCAUUA CAGAGUGGAA AUACCUGUUC CCAUCC;

wherein under stringent hybridization assay conditions said hybridization assay probe hybridizes with nucleic acid from *Ureaplasma urealyticum* to form a probe:target hybrid and does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication that Ureaplasma may be present in said sample.

59. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33.

60. The method of claim 59, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 1: TCATITGACTT GGTGAGGCCAT TACCTCAC,

SEQ ID NO: 3: GCCGTGTCTC AGTCCCATTG TGGCTGTTCT,

SEQ ID NO: 64: UCAUUGACUU GGUGAGCCAU UACCUAC,

SEQ ID NO: 65: GTGAGGTAAT GGCTCACCAA GTCAATGA,

SEQ ID NO: 66: GUGAGGUAAU GGCUCACCAA GUCAAUGA,

SEQ ID NO: 67: GCCGUGUCUC AGUCCCAUUG UGGCUGUUCU,

SEQ ID NO: 68: AGAACAGCCA CAATGGGACT GAGACACGGC, and

SEQ ID NO: 69: AGAACAGCCA CAAUGGGACU GAGACACGGC.

61. A method for detecting the presence of Ureaplasma in a sample and distinguishing said Ureaplasma from *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis*, comprising the steps of:

a) providing to said sample a hybridization assay probe comprising a detection nucleotide base sequence

selected from the group consisting of: SEQ ID NO: 5, SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36; wherein under stringent hybridization assay conditions said hybridization assay probe hybridizes with nucleic acid from *Ureaplasma urealyticum* to form a probe-target hybrid and does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication that *Ureaplasma* may be present in said sample;

wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 4: ATATAAAAGA ACTTTACAAT CTATAAGACC TTTCATCGTTC ACGCGGC,

SEQ ID NO: 6: GGCACATAGT TAGCCGATAC TTATTCAAAT GGTAACAGTCA AA,

SEQ ID NO: 70: AUUAAAAAGA ACUUUACAAU

CUUAAGACC UUCAUCGUUC ACGCGGC,

SEQ ID NO: 71: GCCGCGTGAA CGATGAAGGT

CTTATAGATT GTAAAGTTCT TTTATAT,

SEQ ID NO: 72: GCCCGGUGAA CGAUGAAGGU

CUUAUAGAUU GUAAAGUUCU UUUUAU,

SEQ ID NO: 73: GGCACAUAGU UAGCCGAUAC

UUAUUCAAAU GGUACAGUCA AA,

SEQ ID NO: 74: TTTGACTGTA CCATTTGAAT

AAGTATCGGC TAACTATGTG CC, and

SEQ ID NO: 75: UUUGACUGUA CCAUUUGAAU

AAGUAUCGGC UAACUAUGUG CC.

62. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39.

63. The method of claim 62, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 7: CCTGCGCTCG TTTTACGCC AGTAAATCCG GATAACGC,

SEQ ID NO: 9: CCTACTACAC TCTAGGTTTA CAGTTTTGA TACAGCTAGA,

SEQ ID NO: 40: CCUACUACAC UCUAGGUUA CAGUUUUUGA UACAGCUAGA,

SEQ ID NO: 41: TCTAGCTGTA TCAAAAACGT TAAACCTAGA GTGTAGTAGG,

SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG UAAACCUAGA GUGUAGUAGG,

SEQ ID NO: 76: CCUGCGCUCG UUUUACGCC AGUAAAUCCG GAUAAACGC,

SEQ ID NO: 77: GCGTTATCCG GATTACTGG GCG-TAAAACG AGCGCAGG, and

SEQ ID NO: 78: GCGUUAUCCG GAUUUACUGG GCGUAAAACG AGCGCAGG.

64. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42.

65. The method of claim 64, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 8: CGTTAACAT CTAGATTAA TAC- CAAACATT ACAAACCCG,

SEQ ID NO: 10: GCCTTCGCCA CCGGTGTTCT TCCATATATC TA,

SEQ ID NO: 37: CGUUAAGCAU CUAGAUUUAU UACCAAACUU ACAAACCCG,

SEQ ID NO: 38: CGGGTTTGTA AGTTGGTAT TAAATCTAGA TGCTTAACG,

SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUAU UAAAUCUAGA UCCUUAACG,

SEQ ID NO: 79: GCCUUCGCCA CCGGUGUUCU UCCAUAUAUC UA,

SEQ ID NO: 80: TAGATATATG GAAGAACACC GGTGGCGAAG GC, and

SEQ ID NO: 81: UAGAUUAUAG GAAGAACACC GGUGGCGAAG GC.

66. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45.

67. The method of claim 66, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 10: GCCTTCGCCA CCGGTGTTCT TCCATATATC TA,

SEQ ID NO: 12: CTAATCCTAT TTGCTCCCCA CACTTCGAG CCTAACG,

SEQ ID NO: 79: GCCUUCGCCA CCGGUGUUCU UCCAUAUAUC UA,

SEQ ID NO: 80: TAGATATATG GAAGAACACC GGTGGCGAAG GC,

SEQ ID NO: 81: UAGAUUAUAG GAAGAACACC GGUGGCGAAG GC,

SEQ ID NO: 82: CUAUUCUAU UUGCUCCCCA CACUUUCGAG CCUAGC,

SEQ ID NO: 83: GCTTAGGCTC GAAAGTGTGG GGAGCAAATA GGATTAG, and

SEQ ID NO: 84: GCUUAGGCUC GAAAGUGUGG GGAGCAAUAU GGAAUAG.

68. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 20, SEQ ID NO: 52, SEQ ID NO: 53, and SEQ ID NO: 54.

69. The method of claim 68, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 19: TAGCACGTTT GCAGCCCTAG ATATAAGGGG CATGATG,

SEQ ID NO: 21: CGAATTGCAG CCCTCTATCC GAACTGAGAC TAACTTTTC TG,

SEQ ID NO: 97: UAGCACGUU GCAGCCCUAG AUUAAGGGG CAUGAUG,

SEQ ID NO: 98: CATCATGCC CTTATATCTA GGGCTGCAAAG CGTGTCA,

SEQ ID NO: 99: CAUCAUGCCC CUUAUAUCUA GGGCUGCAA CGUGCUA,

SEQ ID NO: 100: CGAAUUGCAG CCCUCUAUCC GAACUGAGAC UAACUUUUC UG,

SEQ ID NO: 101: CAGAAAAAGT TAGTCTCAGT TCGGATAGAG GGCTGCAATT CG, and,

SEQ ID NO: 102: CAGAAAAAGU UAGUCUCAGU UCGGAUAGAG GGCUGCAAUU CG.

70. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 22, SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 57.

71. The method of claim 70, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 24: GGAACAGGT A TTICCACTCT  
GATATGATCA CTAC,  
SEQ ID NO: 25: GCGTAGCGAT GACCTATTTC  
ACTTGC,  
SEQ ID NO: 103: GGAACAGGU A UUCCACUCU  
GAUAUGAUCA CUAC,  
SEQ ID NO: 104: GTAGTGATCA TATCAGAGTG  
GAAATACCTG TTCC,  
SEQ ID NO: 105: GUAGUGAUCA UAUCAGAGUG  
GAAAUCUG UUCC,  
SEQ ID NO: 106: GCGUAGCGAU GACCBAUUUU  
ACUUGC,  
SEQ ID NO: 107: GCAAGTAAAA TAGGTCATCG  
CTACGC, and  
SEQ ID NO: 108: GCAAGUAAAA UAGGUCAUCG  
CUACGC.

72. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 23, SEQ ID NO: 58, SEQ ID NO: 59, and SEQ ID NO: 60.

73. The method of claim 72, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 26: GGATGGAAC AGGTATTCC  
ACTCTGATAT GATCAC,  
SEQ ID NO: 27: GCGTAGCGAT GACCTATTTC ACT-  
TGCCTA TTT,  
SEQ ID NO: 109: GGAUGGGAAC AGGUAUUCC  
ACUCUGAUU GAUCAC,  
SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA  
ATACCTGTT CCATCC,  
SEQ ID NO: 111: GUGAUCAUAU CAGAGUGGAA  
AUACCUGUUC CCAUCC,  
SEQ ID NO: 112: GCGUAGCGAU GACCBAUUUU  
ACUUGCGCUA UUUU,  
SEQ ID NO: 113: AAAATAGCGC AAGTAAAAATA  
GGTCATCGCT ACGC, and  
SEQ ID NO: 114: AAAAUAGCGC AAGUAAAAUA  
GGUCAUCGCU ACGC.

74. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63.

75. The method of claim 74, wherein said method uses one or more helper probes consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 28: GAGATCAACG GATTAAGGCC TCT-  
TATCAGC TACCCGTTGC TTATCGCAGA  
TTAGCACG,  
SEQ ID NO: 30: CACTTCACCA GGTATCGCTCTGTT-  
TAAACTA TGAATTCAATT TATA,  
SEQ ID NO: 115: GAGAUCAACG GAUUAAGCC  
UCUUAUCAGC UACCCGUUGC UUAUCGCAGA  
UUAGCACG,  
SEQ ID NO: 116: CGTGTCAATC TGCGATAAGC  
AACGGGTAGC TGATAAGAGG CTITTAATCCG  
TTGATCTC,  
SEQ ID NO: 117: CGUGCUAAUC UGCGAUAGC  
AACGGGUAGC UGAUAAGAGG CUUUAUCCG  
UUGAUCUC,

SEQ ID NO: 118: CACUUCACCA GGUAUCGCUC  
UGUUAACUA UGAAUUCAUU UAUU,

SEQ ID NO: 119: TATAATGAA TTCATAGTTT  
AACAGAGCGA TACCTGGTGA AGTG, and

SEQ ID NO: 120: UAUAAAUGAA UUCAUAGUUU  
AACAGAGCGA UACCUGGUGA AGUG.

76. The method of claim 58, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of SEQ ID NO: 26, SEQ ID NO: 109, SEQ ID NO: 110, and SEQ ID NO: 111.

77. The method of any one of claims 59, 60, and 61-76, wherein said hybridization assay probe consists of said detection nucleotide base sequence and one or more reporter groups.

78. A method for specifically detecting the presence of *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2 comprising the steps of:

a) contacting a sample with a hybridization assay probe able to hybridize under stringent hybridization assay conditions to a *Ureaplasma urealyticum* biotype specific target nucleic acid sequence to form a probe:target hybrid with either *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2 nucleic acid, wherein said hybridization assay probe does not hybridize to nucleic acid from both *Ureaplasma urealyticum* biotype 1 and *Ureaplasma urealyticum* biotype 2 under said stringent hybridization assay conditions to form a detectable probe:non-target hybrid, said *Ureaplasma urealyticum* biotype specific target nucleic acid sequence being selected from the group consisting of:

SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,  
SEQ ID NO: 127: CAACACCGAC CCAUUCGG,  
SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG,  
and

SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG;  
provided that under said stringent hybridization conditions said probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable probe:non-target hybrid; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication of the presence of *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2.

79. The method of claim 78, wherein said target nucleic acid sequence is either SEQ ID NO: 126 or SEQ ID NO: 136.

80. The method of claim 78, wherein said target nucleic acid sequence is either SEQ ID NO: 127 or SEQ ID NO: 137.

81. A method for specifically detecting the presence of *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2 comprising the steps of:

a) contacting a sample with a hybridization assay probe able to hybridize under stringent hybridization assay conditions with either *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2 nucleic acid to form a detectable probe:target hybrid, wherein said hybridization assay probe does not hybridize to nucleic acid from both *Ureaplasma urealyticum* biotype 1 and *Ureaplasma urealyticum* biotype 2 under said stringent hybridization assay conditions to form said detectable probe:non-target hybrid, said hybridization assay probe

comprising a detection nucleotide base sequence selected from the group consisting of:  
SEQ ID NO: 121: CAACACCGAC TCGTTCGAGC,  
SEQ ID NO: 122: CAACACCGAC CCATTCGG,  
SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC, 5  
SEQ ID NO: 127: CAACACCGAC CCAUUCGG,  
SEQ ID NO: 131: GCTCGAACGA GTCGGTGTTG,  
SEQ ID NO: 132: CCGAATGGGT CGGTGTTG,  
SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG,

and

SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG;  
provided that under said stringent hybridization conditions said probe does not hybridize to nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae* to form a detectable 15 probe:non-target hybrid; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under said stringent hybridization assay conditions as an indication of the presence 20 of either *Ureaplasma urealyticum* biotype 1 or *Ureaplasma urealyticum* biotype 2.

82. The method of claim 81, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 121: CAACACCGAC TCGTTCGAGC,  
SEQ ID NO: 126: CAACACCGAC UCGUUCGAGC,  
SEQ ID NO: 131: GCTCGAACGA GTCGGTGTTG, and  
SEQ ID NO: 136: GCUCGAACGA GUCGGUGUUG. 30

83. The method of claim 82, further comprising the use of a helper probe consisting of a sequence selected from the group consisting of:

SEQ ID NO: 123: CGACATTAA TGATGATCGT TTACGGTGTG GAC, 35

SEQ ID NO: 125: CCCAGGCACA TCATTAAATG CGTTAGCTA,

SEQ ID NO: 128: CGACAUUUAA UGAUGAUCGU UUACGGUGUG GAC,

SEQ ID NO: 130: CCCAGGCACA UCAUUUAUG CGUUAGCUA, 40

SEQ ID NO: 133: GTCCACACCG TAAACGATCA TCATTAAATG TCG,

SEQ ID NO: 135: TAGCTAACGC ATTAAATGAT GTGCCTGGG, 45

SEQ ID NO: 138: GUCCACACCG UAAACGAUCA UCAUAAAUG UCG, and

SEQ ID NO: 140: UAGCUAACGC AUUAAAUGAU GUGCCUGGG. 50

84. The method of claim 81, wherein said hybridization assay probe comprises a detection nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 122: CAACACCGAC CCATTCGG, 55

SEQ ID NO: 127: CAACACCGAC CCAUUCGG,

SEQ ID NO: 132: CCGAATGGGT CGGTGTTG, and

SEQ ID NO: 137: CCGAAUGGGU CGGUGUUG.

85. The method of claim 84, further comprising the use of a helper probe in said step (a), said helper probe consisting 60 of a sequence selected from the group consisting of:

SEQ ID NO: 124: GCCGACATTAAATGATGATC GTT-TACGGTGTG TGGAC,

SEQ ID NO: 125: CCCAGGCACA TCATTAAATG CGTTAGCTA,

SEQ ID NO: 129: GCCGACAUUU AAUGAUGAUC GUUUACGGUG UGGAC,

SEQ ID NO: 130: CCCAGGCACA UCAUUUAUG CGUUAGCUA,  
SEQ ID NO: 134: GTCCACACCG TAAACGATCA TCATTAAATG TCGGC,  
SEQ ID NO: 135: TAGCTAACGC ATTAAATGAT GTGCCTGGG,  
SEQ ID NO: 139: GUCCACACCG UAAACGAUCA UCAUAAAUG UCGGC, and  
SEQ ID NO: 140: UAGCUAACGC AUUAAAUGAU GUGCCUGGG.

86. The method of any one of claims 82–85, wherein said hybridization assay probe consists of one or more reporter groups and said detection nucleotide base sequence.

87. A method for detecting the presence of *Ureaplasma* in a sample and distinguishing said *Ureaplasma* from *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis*, comprising the steps of:

a) providing to said sample a hybridization assay probe comprising a nucleotide base sequence selected from the group consisting of

SEQ ID NO: 14: CGTCGAGCC GACATTAAAT GATGATCG,

SEQ ID NO: 46: CGUUCGAGCC GACAUUUAAU GAUGAUCG,

SEQ ID NO: 47: CGATCATCAT TAAATGTCGG CTCGAACG, and

SEQ ID NO: 48: CGAUCAUCAU UAAAUGUCGG CUCGAACG; wherein under stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but not with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae*; and

a helper probe consisting of a nucleotide base sequence selected from the group consisting of:

SEQ ID NO: 13: TTACGGTGT GGACTACTAG GGTAT,

SEQ ID NO: 15: GCGTAGCTA CAACACCGAC T,

SEQ ID NO: 85: UUUACGGUGU GGACUACUAG GGUAU,

SEQ ID NO: 86: ATACCTAGT AGTCCACACC GTAAA,

SEQ ID NO: 87: AUACCCUAGU AGUCCACACC GUAAA,

SEQ ID NO: 88: GCGUUAGCUA CAACACCGAC U,

SEQ ID NO: 89: AGTCGGTGT GTAGCTAACCG C, and

SEQ ID NO: 90: AGUCGGUGUU GUAGCUAACCG C; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under stringent hybridization assay conditions as an indication that *Ureaplasma* may be present in said sample.

88. The method of claim 87, wherein said hybridization assay probe consists of one or more reporter groups and a nucleotide base sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48.

89. A method for detecting the presence of *Ureaplasma* in a sample and distinguishing said *Ureaplasma* from *Mycoplasma genitalium*, *Mycoplasma pneumoniae*, and *Mycoplasma hominis* comprising the steps of:

a) providing to said sample a hybridization assay probe comprising a nucleotide base sequence selected from the group consisting of

SEQ ID NO: 17: GCGTCGCAAT AGATGTCAAA  
CCTAG,  
SEQ ID NO: 49: GCGUCGCAAU AGAUGUCAAA  
CCUAG,  
SEQ ID NO: 50: CTAGGTTGA CATCTATTGC 5  
GACGC, and  
SEQ ID NO: 51: CUAGGUUUGA CAUCUAUUGC  
GACGC; wherein under stringent hybridization assay conditions said hybridization assay probe forms a detectable probe:target hybrid with *Ureaplasma urealyticum* nucleic acid, but not with nucleic acid from *Mycoplasma genitalium*, *Mycoplasma hominis* and *Mycoplasma pneumoniae*; and a helper probe consisting of a nucleotide base sequence selected from the group consisting of:  
SEQ ID NO: 16: GTAAGGTTCT ACGTGTATTG  
TCAAATTAAAG CAAACATGCTC CACCAC,  
SEQ ID NO: 18: CGACAACCAT GCACCACCTG  
TCATATTGTT AACCTCAAC,  
SEQ ID NO: 91: GUAGGUUCU ACGUGUUAUUG 10  
UCAAAAUUAAG CAAACUGC CACCAC,  
SEQ ID NO: 92: GTGGTGGAGC ATGTTGCTTA  
ATTGACAAT ACACGTAGAA CCTTAC,  
SEQ ID NO: 93: GUGGUGGAGC AUGUUGCUUA 15  
AUUUGACAAU ACACGUAGAA CCUUAC,  
SEQ ID NO: 94: CGACAACCAU GCACCACCG  
UCAUAUTGUU ACCUCAAC,  
SEQ ID NO: 95: GTTGAGGTTA ACAATATGAC  
AGGTGGTGCA TGGTTGTCG, and  
SEQ ID NO: 96: GUUGAGGUUA ACAAAU AUGAC 20  
AGGUGGUGCA UGGUUGUCG; and

b) employing said stringent hybridization assay conditions and detecting the presence of said detectable probe:target hybrid formed under stringent hybridization assay conditions as an indication that *Ureaplasma* may be present in said sample.

90. The method of claim 89, wherein said hybridization assay probe consists of one or more reporter groups and a nucleotide base sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 49, SEQ ID NO: 50, and SEQ ID NO: 51.

91. A hybridization assay probe 10 to 50 nucleotides in length comprising an oligonucleotide sufficiently complementary to a *Ureaplasma urealyticum* target nucleic acid sequence to form a detectable probe:target hybrid with said *Ureaplasma urealyticum* target nucleic acid sequence under stringent hybridization assay conditions, wherein said *Ureaplasma urealyticum* target nucleic acid sequence is selected from the group consisting of:

SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC G,  
SEQ ID NO: 33: CGCGUAGCUG UACUGAGAGG U,  
SEQ ID NO: 37: CGUUAAAGCAU CUAGAUUUAA 55  
UACCAAACUU ACAAACCCG,  
SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUAU  
UAAAUCUAGA UGCUUAAACG,  
SEQ ID NO: 40: CCUACUACAC UCUAGGUAAA  
CAGUUUUUGA UACAGCUAGA,  
SEQ ID NO: 42: UCUAGCUGUA UCAAAAACUG 60  
UAAAACCUAGA GUGUAGUAGG,  
SEQ ID NO: 43: GUCAGUGUA GUCCAAGUUG GC,  
SEQ ID NO: 45: GCCAACUUUG ACUAUCACUG AC,  
SEQ ID NO: 55: GCUAUUUUCG GCUCUAGAGU 65  
GCUUUGACUUC UGUGUUCGGG AUG,  
SEQ ID NO: 57: CAUCCCGAAC ACAGAAGUCA  
AGCACUCUAG AGCCGAAAAU AGC,

SEQ ID NO: 58: CGGCUCUAGA GUGCUUGACU  
UCUGUGUUCG,  
SEQ ID NO: 60: CGAACACAGA AGUCAAGCAC  
UCUAGAGCCG,  
SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA  
GACUGAGUUU CCUCAUUUCG,  
SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU  
AAUGAGAAUU AGAUTUACUG,  
SEQ ID NO: 109: GGAUGGGAAC AGGUAUUUC 10  
ACUCUGAUU GAUCAC, and  
SEQ ID NO 111: GUGAUCAUUA CAGAGUGGAA  
AUACCUGUCC CCAUCC;  
wherein under said stringent hybridization assay conditions said hybridization assay probe does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma hominis*.

92. The hybridization assay probe of claim 91, wherein said hybridization assay probe also does not form said detectable probe:non-target hybrid with nucleic acid from *Mycoplasma genitalium* and *Mycoplasma pneumoniae*.

93. The hybridization assay probe of claim 91, wherein said hybridization assay probe also does not form said detectable probe:non target hybrid with nucleic acid from *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma capricolum*, *Mycoplasma lipophilum*, and *Mycoplasma salivarium*.

94. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 31 and SEQ ID NO: 33.

95. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 37 and SEQ ID NO: 39.

96. The hybridization assay probe of claim 91, wherein said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 40 and SEQ ID NO: 42.

97. The hybridization assay probe of claim 91, wherein 40 said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 43 and SEQ ID NO: 45.

98. The hybridization assay probe of claim 91, wherein 45 said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 55 and SEQ ID NO: 57.

99. The hybridization assay probe of claim 91, wherein 50 said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 58 and SEQ ID NO: 60.

100. The hybridization assay probe of claim 91, wherein 55 said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 61 and SEQ ID NO: 63.

101. The hybridization assay probe of claim 91, wherein 60 said target *Ureaplasma urealyticum* nucleic acid sequence is selected from the group consisting of SEQ ID NO: 109 and SEQ ID NO: 111.

102. A probe mix comprising:

a) a hybridization assay probe for detecting *Ureaplasma* under stringent hybridization assay conditions which is 65 10 to 50 nucleotides in length and comprises a nucleotide base sequence selected from the group consisting of

SEQ ID NO: 2: ACCTCTAGT ACAGCTACGC G,  
SEQ ID NO: 8: CGTTAAGCAT CTAGATTAA TAC-  
CAAACCTT ACAAAACCG,

SEQ ID NO: 9: CCTACTACAC TCTAGGTTA  
CAGTTTTGA TACAGCTAGA,  
SEQ ID NO: 11: GTCAGTGATA GTCCAAGTTG  
GC,  
SEQ ID NO: 20: CGAITTTGCA GCAGTTGTA 5  
TTAGCCATTG,  
SEQ ID NO: 22: GCTATTTCG GCTCTAGAGT  
GCTTGACTTC TGTGTTGGG ATG,  
SEQ ID NO: 23: CGGCTCTAGA GTGCTGACT  
TCTGTGTTCG,  
SEQ ID NO: 26: GGATGGGAAC AGGTATTTC  
ACTCTGATAT GATCAC,  
SEQ ID NO: 29: CAGTAATCTA ATTCTCATTA  
GAETGAGTTT CCTCAATTG,  
SEQ ID NO: 31: ACCUCUCAGU ACAGCUACGC 15  
G,  
SEQ ID NO: 32: CGCGTAGCTG TACTGAGAGG T,  
SEQ ID NO: 33: CGGUAGCUG UACUGAGAGG  
U,  
SEQ ID NO: 37: CGUUUAAGCAU CUAGAUUUAA 20  
UACCAAACUU ACAAAACCG,  
SEQ ID NO: 38: CGGGTTTGTA AGTTTGGTAT  
TAAATCTAGA TGCTTAACG,  
SEQ ID NO: 39: CGGGUUUGUA AGUUUGGUUA 25  
UAAAUCUAGA UGCUUUAACG,  
SEQ ID NO: 40: CCUACUACAC UCUAGGUUA  
CAGUUUUUGA UACAGCUAGA,  
SEQ ID NO: 41: TCTAGCTGA TCAAAAAACTG  
TAAACCTAGA GTGTAGTAGG,  
SEQ ID NO: 42: UCUAGCUGUA UCAAAACUG 30  
UAAAACCUAGA GUGUAGUAGG,  
SEQ ID NO: 43: GUCAGUGUA GUCCAAGUUG  
GC,  
SEQ ID NO: 44: GCCAACTTGG ACTATCACTG  
AC, 35  
SEQ ID NO: 45: GCCAACUUUGG ACUAUCACUG  
AC,  
SEQ ID NO: 52: CGAUUUUGCA GCAGUUUGUA  
UUAGCCAUUG,  
SEQ ID NO: 53: CAATGGCTAA TACAAACTGC 40  
TGCAAAATCG,  
SEQ ID NO: 54: CAAUGGCCUA UACAAACUGC  
UGCAAAACUG,  
SEQ ID NO: 55: GCUAUUUUCG GCUCUAGAGU  
GCUUGACUUC UGUGUUUUCGG AUG, 45  
SEQ ID NO: 56: CATCCCGAAC ACAGAAGTCA  
AGCACTCTAG AGCCGAAAT AGC,  
SEQ ID NO: 57: CAUCCCGAAC ACAGAAGUCA  
AGCACUCUAG AGCCGAAAAU AGC,  
SEQ ID NO: 58: CGGCUCUAGA GUGCUUGACU 50  
UCUGUGUUCG,  
SEQ ID NO: 59: CGAACACAGA AGTCAAGCAC  
TCTAGACCG,  
SEQ ID NO: 60: CGAACACAGA AGUCAAGCAC  
UCUAGAGCCG, 55  
SEQ ID NO: 61: CAGUAAUCUA AUUCUCAUUA  
GACUGAGUUU CCUCAUUUCG,  
SEQ ID NO: 62: CGAATGAGGA AACTCAGTCT  
AATGAGAAIT AGATACTG,  
SEQ ID NO: 63: CGAAUGAGGA AACUCAGUCU 60  
AAUGAGAAU AGAAUACUG,  
SEQ ID NO: 109: GGAUUGGGAAC AGGUAU-  
UUCC ACUCUGAUUA GAUCAC,  
SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA 65  
ATACCTGTT CCATCC, and  
SEQ ID NO: 111: GUGAUCAUUA CAGAGUGGAA.  
AUACCUGUUC CCAUCC;

wherein under stringent hybridization assay conditions  
said hybridization assay probe forms a detectable probe:  
target hybrid with *Ureaplasma urealyticum* nucleic  
acid, but does not form a detectable probe:non-target  
hybrid with nucleic acid from *Mycoplasma genitalium*,  
*Mycoplasma hominis* and *Mycoplasma pneumoniae*  
under said stringent hybridization assay conditions; and

b) a helper probe.

103. The probe mix of claim 102, wherein said hybrid-  
ization assay probe comprises a nucleotide base sequence  
selected from the group consisting of SEQ ID NO: 22, SEQ  
ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 57, and said  
helper probe comprises a nucleotide base sequence selected  
from the group consisting of:

SEQ ID NO: 24: GGAACAGGTA TTTCACACT  
GATATGATCA CTAC,  
SEQ ID NO: 25: GCGTAGCGAT GACCTATTT  
ACTTGC,  
SEQ ID NO: 103: GGAACAGGUA UUUCACACU  
GAUAUGAUCA CUAC,  
SEQ ID NO: 104: GTAGTGATCA TATCAGAGTG  
GAAATACCTG TTCC,  
SEQ ID NO: 105: GUAGUGAUCA UAUCAGAGUG  
GAAAUACCU UUCC,  
SEQ ID NO: 106: GCGUAGCGAU GACCUAUUU  
ACUUGC,  
SEQ ID NO: 107: GCAAGTAAAA TAGGTCATCG  
CTACGC, and  
SEQ ID NO: 108: GCAAGUAAAA UAGGUCAUCG  
CUACGC.

104. The probe mix of claim 103, wherein said probe mix  
is selected from the group consisting of:

(a) a probe mix comprising:

a hybridization assay probe consisting of one or more  
reporter groups and the nucleotide base sequence of  
either SEQ ID NO: 22 or SEQ ID NO: 55;  
a first helper probe consisting of the nucleotide base  
sequence of either SEQ ID NO: 24 or SEQ ID NO:  
103; and  
a second helper probe consisting of the nucleotide base  
sequence of either SEQ ID NO: 25 or SEQ ID NO:  
106; and

(b) a probe mix comprising:

a hybridization assay probe consisting of one or more  
reporter groups and the nucleotide base sequence of  
either SEQ ID NO: 56 or SEQ ID NO: 57;  
a first helper probe consisting of the nucleotide base  
sequence of either SEQ ID NO: 104 or SEQ ID NO:  
105; and  
a second helper probe consisting of the nucleotide base  
sequence of either SEQ ID NO: 107 or SEQ ID NO:  
108.

105. The probe mix of claim 102, wherein said hybrid-  
ization assay probe comprises a nucleotide base sequence  
selected from the group consisting of SEQ ID NO: 23, SEQ  
ID NO: 58, SEQ ID NO: 59, and SEQ ID NO: 60, and said  
helper probe comprises a nucleotide base sequence selected  
from the group consisting of

SEQ ID NO: 26: GGATGGGAAC AGGTATTTC  
ACTCTGATAT GATCAC,  
SEQ ID NO: 27: GCGTAGCGAT GACCTATTT ACT-  
TGGCTA TTTT,  
SEQ ID NO: 109: GGAUUGGGAAC AGGUAUUUC  
ACUCUGAUUA GAUCAC,  
SEQ ID NO: 110: GTGATCATAT CAGAGTGGAA  
ATACCTGTT CCATCC,

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SEQ ID NO: 111: GUGAUCAUAU CAGAGUGGAA  
AUACCUGUUC CCAUCC,  
SEQ ID NO: 112: GCGUAGCGAU GACCUAULUU  
ACUUGCCUA UUU,  
SEQ ID NO: 113: AAAATAGCGC AAGTAAAATA 5  
GGTCATCGCT ACGC, and  
SEQ ID NO: 114: AAAAUAGCGC AAGUAAAAUA,  
GGUCAUCGU ACGC.

106. The probe mix of claim 105, wherein said probe mix is selected from the group consisting of:

(a) a probe mix comprising:

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 23 or SEQ ID NO: 58;

a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 26 or SEQ ID NO: 109; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 27 or SEQ ID NO: 112; and

(b) a probe mix comprising:

a hybridization assay probe consisting of one or more reporter groups and the nucleotide base sequence of either SEQ ID NO: 59 or SEQ ID NO: 60;

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a first helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 110 or SEQ ID NO: 111; and

a second helper probe consisting of the nucleotide base sequence of either SEQ ID NO: 113 or SEQ ID NO: 114.

107. A hybridization assay probe 10 to 100 nucleotides in length comprising an oligonucleotide sufficiently complementary to a *Ureaplasma urealyticum* target nucleic acid sequence to form a detectable probe:target hybrid with said *Ureaplasma urealyticum* target nucleic acid sequence under stringent hybridization assay conditions, wherein said *Ureaplasma urealyticum* target nucleic acid sequence is SEQ ID NO: 54: CAAUGGCUAA UACAAACUGC 15 UGCAAAAUUCG, and said hybridization assay probe targets at least one nucleotide 5' to "A" at nucleotide position 11 in SEQ ID NO: 54, wherein under said stringent hybridization assay conditions said hybridization assay probe does not form a detectable probe:non-target hybrid with nucleic acid from *Mycoplasma hominis*.

\* \* \* \* \*